OPEN ACCESS

University of Dundee

University of Dundee

# Gloeobacter and the implications of a freshwater origin of Cyanobacteria

Raven, John A.; Sánchez-Baracaldo, Patricia

*Published in:*
Phycologia

*DOI:*
10.1080/00318884.2021.1881729

*Publication date:*
2021

*Licence:*
CC BY-NC-ND

*Document Version*
Publisher's PDF, also known as Version of record

Link to publication in Discovery Research Portal

*Citation for published version (APA):*
Raven, J. A., & Sánchez-Baracaldo, P. (2021). Gloeobacter and the implications of a freshwater origin of Cyanobacteria. *Phycologia*, *60*(5), 402-418. https://doi.org/10.1080/00318884.2021.1881729

**General rights**
Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

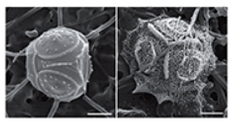
• Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain.
• You may freely distribute the URL identifying the publication in the public portal.

**Take down policy**
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 05. May. 2023

Phycologia

Phycologia



ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uphy20

# *Gloeobacter* and the implications of a freshwater origin of Cyanobacteria

John A. Raven & Patricia Sánchez-Baracaldo

**To cite this article:** John A. Raven & Patricia Sánchez-Baracaldo (2021): *Gloeobacter* and the implications of a freshwater origin of Cyanobacteria, Phycologia, DOI: 10.1080/00318884.2021.1881729

**To link to this article:** https://doi.org/10.1080/00318884.2021.1881729

© 2021 The Author(s). Published with license by Taylor & Francis Group, LLC.

Published online: 07 Apr 2021.

Submit your article to this journal

Article views: 206

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at https://www.tandfonline.com/action/journalInformation?journalCode=uphy20

PHYCOLOGIA
https://doi.org/10.1080/00318884.2021.1881729

OPEN ACCESS Check for updates

# *Gloeobacter* and the implications of a freshwater origin of Cyanobacteria

JOHN A. RAVEN[1,2,3] AND PATRICIA SÁNCHEZ-BARACALDO[4]

[1]*Division of Plant Science, University of Dundee at the James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK*
[2]*Climate Change Cluster, Faculty of Science, University of Technology, Sydney, Sydney, Ultimo 2007, Australia*
[3]*School of Biological Sciences, University of Western Australia, Crawley 6009, Australia*
[4]*School of Geographical Sciences, University of Bristol, Bristol BS8 1SS, UK*

**ABSTRACT**

The earliest branching cyanobacterium, *Gloeobacter*, exhibits a number of ancestral traits including the lack of thylakoids. It occurs epilithically in microbial mats, both subaerially and submerged in low-salinity habitats. These habitats and the absence of thylakoids are associated with the occurrence of membrane-associated photosynthetic processes in the plasma membrane, possibly limiting the rate of both assembly and reassembly of the oxygen-evolving complex, as well as the photosynthetic rate and in vitro growth rate. These factors interact with the occurrence of *Gloeobacter* in mats to constrain productivity in nature. Traits found in living *Gloeobacter*, with the probable time of origin of oxygenic photosynthesis and diversification of cyanobacteria, can be related to the possible role of oxygenic primary productivity and organic carbon burial on land during the early Earth in low-salinity environments around the time of the global oxidation event.

**ARTICLE HISTORY**
Received 29 September 2020
Accepted 23 January 2021
Published online 07 April 2021

**KEYWORDS**
Cyanobacterial mats; Global oxidation event; *Gloeobacter*; Non-marine habitats; Oxygen evolution complex; Photosynthesis

## INTRODUCTION

Charles Darwin, in a letter to Joseph Hooker dated 1 February 1871, wrote the following about the origin of life: "But if (& oh what a big if) we could conceive in some warm little pond with all sorts of ammonia & phosphoric salts, – light, heat, electricity, etc., present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present day such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed (darwinproject.ac.uk/letter/DCP-LETT-7471.xml). This suggestion of aquatic habitats on land, as opposed to in the ocean, has been followed up in a number of papers on the location and mechanism of the origin of life (e.g. Follmann & Brownson 2009; Damer & Deamer 2020; Toner & Catling 2020); however, neither a site nor an associated mechanism, can yet explain life's origin (Kitadai & Maruyama 2018; Duval *et al.* 2020).

Besides the possible role as the location of the origin of life, the land surface and aquatic habitats thereon appear to be the site of origin of cyanobacteria (Blank & Sánchez-Baracaldo 2010; Uyeda *et al.* 2016; Ponce-Toledo *et al.* 2017; Nakov *et al.* 2017; Sánchez-Baracaldo *et al.* 2017a, 2017b; see Blank 2013b).

## HABITAT OF BASAL CYANOBACTERIA

Rippka *et al.* (1974) described *Gloeobacter violaceus* Rippka, J. B. Waterbury & Cohen-Bazire, a cyanobacterium lacking thylakoid that was isolated from a limestone exposure in Switzerland, a low-salinity terrestrial habitat. SSU rRNA sequences showed that *Gloeobacter violaceus* was the basal member among the cyanobacterial sequences sampled (Nelissen *et al.* 1995). Blank & Sánchez-Baracaldo (2010) confirmed this by analysing the small and large subunit of rDNA and 137 protein sequences, and emphasized that *Gloeobacter violaceus* was the earliest branching or basal organism in Cyanobacteria. It is therefore likely that *Gloeobacter* spp. have retained 'primitive' or ancestral traits, and that such traits have undergone little change since being inherited from the common ancestor. It is important to point out that some of these traits might also be apomorphies, or traits that are unique to *Gloeobacter* and not necessarily present in other Cyanobacteria; specially given its long history. Given its phylogenetic position, it is reasonable to infer traits that might have been present in ancestral lineages of Cyanobacteria.

All known strains of *Gloeobacter* grow in low-salinity habitats, which was confirmed by the study of compatible solutes in a number of cyanobacteria by Blank (2013a). Mareš *et al.* (2013a, 2013b, 2013c) showed that *Gloeobacter violaceus* is a widespread terrestrial organism (see also Chrismas *et al.* 2015; Pushkareva *et al.* 2015; Williams *et al.* 2016; Popovic *et al.* 2019), although it has also been found in shallow freshwater cyanobacterial mats (Lionard *et al.* 2012) A second species, *Gloeobacter kīlaueensis* J.W. Saw *et al.*, was isolated as the dominant cyanobacterium in a 5 mm thick epilithic biofilm at the entrance to a lava cave in the Kīlauea Caldeira, Hawai'i (Saw *et al.* 2013). Both *Gloeobacter* species grow photolithotrophically in the 'freshwater' medium BG11 (Andersen *et al.* 2005). Poikilohydric terrestrial photosynthetic organisms are generally subject to episodes of desiccation, while nothing seems to be known of desiccation

**CONTACT** John A. Raven j.a.raven@dundee.ac.uk

© 2021 The Author(s). Published with license by Taylor & Francis Group, LLC.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

tolerance in *Gloeobacter violaceus* (Potts *et al.* 2005; Lüttge 2011). However, *Gloeobacter violaceus* has a gene (glr0712) with 40% similarity to DdrA, a gene from the very dehydration-, UV-, and ionizing radiation-tolerant bacterium *Deinococcus radiodurans* Brooks & Murray (Potts *et al.* 2005; Lüttge 2011) that inhibits nuclease action on single-stranded DNA resulting from damage to the nucleome. As is discussed below, very little is known of UV screening and repair of UV-induced damage in *Gloeobacter* species (Rastogi *et al.* 2014; Pathak *et al.* 2019), although there are studies of the capacity for DNA repair (Cassier-Chauva *et al.* 2016).

Grettenberger *et al.* (2020) described an uncultured cyanobacterium (*candidatus Aurora vandensis*) based on metagenome data, showing that it is a sister clade to *Gloeobacter* spp. *Gloeobacter* plus *candidatus Aurora vandensis* are the closest cyanobacterial relatives of the non-photosynthetic Vampirovibrionia (formerly Melaianabacteria). Based on the presence of the relevant genes, *candidatus Aurora vandensis* has the potential to grow photolithotrophically using oxygenic photosynthesis (Grettenberger *et al.* 2020). The future isolation and culture of *candidatus Aurora vandensis* is important to explore their metabolic and physiological capabilities since the organism is known only from metagenomic studies. The metagenome was obtained from benthic microbial mats from the freshwater (salinity 0.242) epilimnion of the meromictic Lake Vanda in the Antarctic (Huffert & Turner 1972; Sumner *et al.* 2016; Grettenberger *et al.* 2020). The occurrence of *candidatus Aurora vandensis* in benthic microbial mats does not necessarily mean that this cyanobacterium alone can form a microbial mat.

## ADAPTATION OF FRESHWATER CYANOBACTERIA TO LIFE IN SALINE ENVIRONMENTS

Herrmann & Gehringer (2019) examined salinity tolerance of *Gloeobacter violaceus* and the later-evolving *Chrococcidiopsis thermalis* Geitler as an indicator of their potential to establish in estuarine and marine environments following washout from their typical low-salinity habitats. Both *Gloeobacter* and *Chrococcidiopsis* grew in brackish culture media, although more slowly than in freshwater; only *Chrococcidiopsis* grew at seawater salinities, probably related to its ability to synthesize the osmoregulant trehalose (Blank 2013a; Herrmann & Gehringer 2019). Growth periods were 12 days for *Chrococcidiopsis* and 24 days for *Gloeobacter*, so the experiment was not experimental evolution that requires many more generations, as used in experimental evolution studies of salinity adaptation using the freshwater chlorophycean microalga *Chlamydomonas reinhardtii* P.A. Dangeard (Perrineau *et al.* 2014; Lachapelle *et al.* 2015). While experimental evolution has been applied to cyanobacteria, none of these studies have addressed variations in salinity (Leister 2018). The suggestion that the ability to synthesize trehalose is an indicator of salinity tolerance (Blank 2013a; Herrmann & Gehringer 2019) contrasts with an earlier suggestion in a literature review (Hagemann 2011). The review suggests that sucrose and trehalose occur in freshwater cyanobacteria, while glucosylglycerol and glucoosylglycerate are compatible solute osmoregulators in moderately halotolerant (marine) cyanobacteria, and that halophilic cyanobacteria are characterized by glycine betaine and glutamate betaine (Hagemann 2011). However, Pade *et al.* (2012) showed that trehalose is the major compatible solute in the marine diazotrophic *Crocosphaera watsonii* Zehr, R.A. Foster, J.B. Waterbury & Webb, strain WH8501. Another marine diazotrophic cyanobacterium, *Trichodesmium erythreum* Ehrenberger & Gomont, strain MS 101, has N,N,N-trimethylhomoserine as the compatible solute (Pade *et al.* 2016). Some marine cyanobacteria, e.g. picocyanobacterial from the SynPro clade, have in their genome the capacity to synthesize a wide range of compatible solutes potentially enabling them to thrive in both marine and hypersaline environments (Scanlan *et al.* 2009). While some of these strains do not grow in hypersaline habitats, the data suggest that those strains possessing glucosylglycerol, glucosylglycerate and/or glycine betaine have the ability to tolerate environments where salt concentration shows large fluctuations.

In addition to compatible solutes as osmoregulants, there are differences in ion transport processes at the cell membrane of freshwater compared to marine cyanobacteria (Hagemann 2011; Raven & Beardall 2020). Possibly related to these changes in ion transport and ion content are differences in the isoelectric point of proteins in freshwater and marine cyanobacteria (Cabellero-Yeves *et al.* 2018; Cabello-Yeves & Rodriguez-Valera 2019). As with the other (non-cyanobacterial) bacteria tested, the proteins of a freshwater species of *Synechococcus* have a higher mean isoelectric point (higher ratio of basic to acidic amino-acid residues) than the proteins of a marine *Synechococcus* (Cabello-Yeves & Rodriguez-Valera 2019). Cabello-Yeves & Rodriguez-Valera (2019) suggest that such large-scale changes in amino-acid composition would take many generations of adaptation.

The effect on micro-organisms of increased salinity has similarities to the effect of desiccation, and the terrestrial habitats of *Gloeobacter violaceus* are subject to drying episodes (Potts *et al.* 2005); however, as mentioned above, nothing is known of desiccation tolerance in this cyanobacterium (Potts *et al.* 2005; Lüttge 2011).

## COMPARISON OF STRUCTURE AND BIOGENESIS OF PHOTOSYNTHETIC MEMBRANES IN *GLOEOBACTER* AND OTHER CYANOBACTERIA

Except for *Gloeobacter*, all known cyanobacteria have their membrane-associated reactions of photosynthesis and respiratory energy transduction in thylakoids, although some respiratory reactions also occur in the plasma membrane (Mullineaux 2014; Liu 2016; Mullineaux & Liu 2020). Thylakoids of cyanobacteria were thought to be related biosynthetically to the plasma membrane via the 'convergence membrane' that closely approaches the plasma membrane and is studded with ribosomes but not phycobilisomes (Vothknecht & Westhoff 2001; Rast *et al.* 2019) in many cyanobacteria. Rast *et al.* (2019; see also Stengel *et al.* 2012) suggest that the 'convergence membrane' is a major site of thylakoid integral membrane protein synthesis, although the convergence membrane may be absent naturally or as a result of downregulation of the protein curt1 (Heinz *et al.* 2016). Furthermore, Mahbub *et al.* (2020) found that newly synthesized PSI and PSII were inserted over the

whole thylakoid surface, although there is evidence that the thylakoid lipid digalactosyldiacylglyceride (DGDG) is synthesized in the plasma membrane of thylakoid-containing cyanobacteria (Selão *et al.* 2014). Overall, there seems to be no role of the plasma membrane in synthesis of the protein components of thylakoids, separating the site of synthesis of photosynthetic membrane proteins in *Gloeobacter* from that in other, thylakoid-containing, cyanobacteria.

Vothknecht & Westhoff (2001), Westphal *et al.* (2001), and Heidrich *et al.* (2017) showed that inactivation of the Vipp1 gene prevented thylakoid production. *Gloeobacter violaceus*, but not *candidatus Aurora vandensis*, also has a homologue of the Vipp 1 protein found in all other oxygenic photosynthetic organisms (Theis *et al.* 2018; Mareš *et al.* 2019; Grettenberger *et al.* 2020). Vipp 1 is involved in thylakoid synthesis, associating especially with curved regions; however, the *Gloeobacter violaceus* protein lacks the approximately 30 amino-acid extension found in other oxygenic photosynthetic organisms but not in most of the ancestors of Vipp in non-cyanobacterial bacteria (Theis *et al.* 2018). However, the role of the truncated Vipp homologue in *Gloeobacter* is not yet known.

Membrane curvature at the margin is an essential feature of thylakoids, with a prediction that monogalactosyldiacylglyceride (MGDG), with a large area of the fatty acid chain relative to the hydrophilic head group, accumulates on the concave, lumenal side of the curved membrane (Murphy 1982). However, determination of the distribution of MGDG between the convex, outer (cytosol- or stroma-facing) and concave, inner (lumen-facing) leaflets of the thylakoid shows that MGDG is enriched in the *outer* leaflet (Siegenthaler *et al.* 1988; Rawyler *et al.* 1987). Moreover, MGDG occurs in the thylakoid-less *Gloeobacter* (Selstam & Campbell 1996). More significantly, CurT, a homologue of the CURVATURE PROTEIN of *Arabidopsis thaliana* (Linnaeus) Heynh is essential for thylakoid architecture of *Synechocystis* sp. strain PCC 6803 and one of the at least 5 variants is enriched in the thylakoid margin; a variant occurs in lower abundance in the plasma membrane (Heinz *et al.* 2016). CurT is also involved in thylakoid biogenesis (Heinz *et al.* 2016). However, CurT is absent from several basal lineages of thylakoid-containing cyanobacteria, and from *Gloeobacter* (Mareš *et al.* 2019). For instance, CurT is missing from *Gloeomargarita*, the closest living relative of the ancestor of eukaryote plastids (Nakov *et al.* 2017; Ponce-Toledo *et al.* 2017; Sánchez-Baracaldo *et al.* 2017a, 2017b), all of which are believed to contain CurT. One possible explanation of this is that the cyanobacterial chloroplast ancestor contained CurT, and that CurT was lost in the *c.* 1.8 Ga leading to the extant *Gloeomargarita*. Another possibility is that CurT was not present in the cyanobacterial ancestor, and evolved, or was obtained by horizontal gene transfer from an unknown source, in the last chloroplast common ancestor. These considerations, however, do not help to indicate whether *Gloeobacter* is ancestrally thylakoid-free or has lost its thylakoids.

## IMPLICATIONS OF LOCATION OF MEMBRANE-ASSOCIATED COMPONENTS OF PHOTOSYNTHESIS, RESPIRATION, AND $H^+$ PUMPING RHODOPSIN, IN THE PLASMA (I.E. CELL OR INNER) MEMBRANE OF *GLOEOBACTER*: COMPARISON WITH OTHER CYANOBACTERIA

### Background

*Gloeobacter* is unique among cultured cyanobacteria in having the membrane-associated components of photosynthesis, as well as those of respiration, in the cell (i.e. inner or plasma) membrane rather than in thylakoids (Rippka *et al.* 1974; Lea-Smith *et al.* 2010; Rexroth *et al.* 2011; Mullineaux 2014; Liu 2016; Mareš *et al.* 2019). In addition to photosynthetic and respiratory electron transporters, the plasma membrane of *Gloeobacter* houses the transporters required for nutrient acquisition, including the non-carboxysomal components of the β-cyanobacterial $CO_2$-concentrating mechanism, ion regulation, and a light-powered rhodopsin proton pump (Rexroth *et al.* 2011; Burnap *et al.* 2015). These co-locations have numerous implications for the functioning of the *Gloeobacter* cell relative to that of other cyanobacteria.

### Membrane-associated components of photosynthesis and respiration

There seem to be no data on the thylakoid membrane surface area per unit plasma membrane area in cyanobacteria, but the electron micrographs of a wide phylogenetic variety of cyanobacterial taxa in Mareš *et al.* (2019) suggest that it is always greater than 2. Thus, for a given cell volume and shape, the area of membrane available for the membrane-associated components of photosynthesis and respiration (typically

**Table 1.** Comparison of chlorophyll *a* per cell, net photosynthetic rate per unit chlorophyll, net photosynthetic rate per cell, and thylakoid area per plasma membrane area in *Gloeobacter, Synechocystis* and *Synechococcus*. Data from Kihara *et al.* (2014). Thylakoid area per plasma membrane area is an upper limit, as indicated by ≤, because the spherical surface area which is the maximum area of the thylakoids is less than that of the plasma membrane because the thylakoids are interior to the plasma membrane.

| Organism[a] | Mole chlorophyll *a* $cell^{-1}$ | Net photosynthetic rate (mole $O_2$ mole chlorophyll $a^{-1}$) $s^{-1}$ | Net photosynthetic rate (mole $O_2$ $cell^{-1} \cdot s^{-1}$) | Thylakoid area (plasma membrane area)$^{-1}$ |
|---|---|---|---|---|
| *Gloeobacter* | $1.27\ 10^{-16}$ | 0.031 | $0.62\ 10^{-18}$ | 0 |
| *Synechocystis* | $1.4\ 10^{-16}$ | 0.061 | $1.4\ 10^{-18}$ | ≤ 6 |
| *Synechococcus* | $0.207\ 10^{-16}$ | 0.057 | $2.7\ 10^{-18}$ | ≤ 4 |

[a]Kihara *et al.* (2014) do not give strain numbers for the genera of Cyanobacteria modelled.

found in thylakoid membrane) in *Gloeobacter* is less than that in the two cyanobacteria with thylakoids (Table 1). This is exacerbated by the occurrence of nutrient acquisition and ion regulation transporters and channels, and ion-pumping rhodopsin, as well as membrane-associated photosynthetic and respiratory components, in the plasma membrane of *Gloeobacter*, with some degree of lateral segregation of functions (Rexroth *et al.* 2011). This suggests that the chlorophyll per unit plasma membrane area in *Gloeobacter* is less than that in the thylakoids of other cyanobacteria, since the thylakoid membrane has fewer functions than the plasma membrane of *Gloeobacter*. However, the limited data available suggest that this is not the case (Table 1). Furthermore, the maximum rate of net photosynthesis per cell is not correlated with the presence and number of thylakoids (Table 1).

Among the problems with the analysis in Table 1 is that the data on chlorophyll *a* per cell is not paralleled by measurements of cell dimensions in the literature cited. Instead, following Kihara *et al.* (2014), it is assumed that the equivalent spherical cell radius to the plasma membrane is 0.5 $10^{-6}$ m, and it is known that cell size of the three genera varies genotypically and phenotypically (e.g. Binder & Liu 1998; Sosik *et al.* 2003; Du *et al.* 2016; Ruan & Giordano 2017; Mareš *et al.* 2019; Zavřal *et al.* 2019). Such genotypic and phenotypic variations also occur for chlorophyll per cell (Liu *et al.* 1999; Six *et al.* 2004; Luimetra *et al.* 2019). For *Synechococcus* the genotypic and phenotypic variation in plasma membrane area per cell ranges from 3.1 $10^{-12}$ to 21 $10^{-12}$ $m^2$ (Sosik *et al.* 2003; Ruan & Giordano 2017) as compared to the assumed 12.6 $10^{-12}$ $m^2$ from Kihara *et al.* (2014). Clearly, further work is needed to establish the effect of the presence of thylakoids on chlorophyll per cell normalized to plasma membrane area, using chlorophyll and area data from the same organisms and growth conditions.

It is likely that the absence of thylakoids decreases the package effect (self-shading) of photosynthetic pigments comparing cells of the same size; however, the package effect is very small in cells of small effective spherical radii such as those of *Gloeobacter* (Raven 1984). A small-package effect means that less time is taken to recoup the energy cost of synthesis of pigment-protein light-harvesting complexes in terms of photons harvested and used in photochemistry in a given light field (Raven 1984). However, the tendency of cells of *Gloeobacter* to form aggregates in culture (Selstam & Campbell 1996; Sicora *et al.* 2008; Mimuro *et al.* 2011; Saw *et al.* 2013), as is the case in natural populations in mats (Saw *et al.* 2013; Mareš *et al.*2013a), would increase the package effect.

## Implications of co-location of $H^+$-pumping rhodopsin and redox-driven $H^+$ pumps

The occurrence of $H^+$-pumping rhodopsin in the *Gloeobacter* plasma membrane means that, in the light, there are three categories of $H^+$ efflux pumps: the photosynthetic redox reactions, any residual (non light-inhibited) respiratory redox reactions, and rhodopsin (Raven 2009; Rexroth *et al.* 2011). Choi *et al.* (2014) quantified the contribution of these pumps to the overall proton efflux by measuring changes in external pH. This was done in a medium of known buffer capacity, around cells of *Gloeobacter* illuminated at a number of wavelengths in the presence and absence of the photosystem II inhibitor DCMU i.e. 3-(3,4-dichlorophenyl)-1,1-dimethyl urea. The inhibitor prevents $H^+$-pumping linear electron flow, but not $H^+$-pumping cyclic electron flow involving PSI and by rhodopsin (Choi *et al.* 2014).

The action spectrum in the absence of DCMU shows involvement of chlorophyll *a* and rhodopsin, with no clear evidence of involvement of photons absorbed by the phycobilins phycoerythrin and phycocyanin, with excitation energy transferred to chlorophyll *a*, in energized $H^+$efflux (Choi *et al.* 2014, fig. 5). Comparison with the absorption spectrum of whole cells and isolated membranes of *Gloeobacter*, dominated by chlorophyll *a* and phycobilins (Choi *et al.* 2014, fig. 4), suggests more effective use of photons absorbed by rhodopsin rather than pigments supplying excitation energy to PSI and PSII. In the presence of DCMU, rhodopsin dominates the action spectrum i.e. Choi *et al.* 2014 of $H^+$ efflux, although there is evidence of energization of some $H^+$ efflux of using of photons absorbed by chlorophyll *a*, presumably using photosystem I-driven cyclic electron flow (not yet fully characterized in *Gloeobacter*). Furthermore, the net $H^+$ efflux is lower in the presence of DCMU is less than in the minus DCMU control (Choi *et al.* 2014, fig. 5).

However, the measurements of net proton efflux, presumably obtained by back-titration to the pH of the medium, cannot be related to the biomass of *Gloeobacter* with the data provided. Furthermore, since no indication is given of using an inorganic C-free bathing solution in the experiments (Choi *et al.* 2014), the pH changes could be influenced by net $CO_2$ consumption in the absence of DCMU, and by residual respiratory $CO_2$ production in the DCMU treatment. For the experiments in the absence of DCMU but presence of $CO_2$, the requirement in $CO_2$ fixation, energized by photons absorbed by chlorophyll *a* and phycobilins, for ATP generated by $H^+$ re-entry through the $CF_OCF_1$ ATP synthase, would bias net $H^+$ efflux in favour of photons absorbed by rhodopsin.

Choi *et al.* (2014) also measured ATP content of cells illuminated in the presence or absence of DCMU. However, the ATP measurements (Choi *et al.* 2014, fig. 5) do not indicate the rate of ATP synthesis; the steady-state content of ATP is a function of the rate of both ATP synthesis and ATP consumption, e.g. in $CO_2$ assimilation in the absence of DCMU.

## Implications of the periplasmic occurrence of the $O_2$-evolving complex for intracellular $O_2$ concentration during photosynthesis

A further effect of the location of membrane-associated photosynthetic reactions in the plasma membrane of *Gloeobacter*, with $O_2$ production on the periplasmic side of the plasma membrane, is the excess of $O_2$ in photosynthesizing cells over that in the medium (Kihara *et al.* 2014). In all other cultured cyanobacteria, and in all photosynthetically competent eukaryotes, $O_2$ production occurs internal to the plasmalemma. In all organisms with thylakoids, $O_2$ is generated in the thylakoid lumen. This means that in cyanobacteria

$O_2$ has to diffuse through the thylakoid membrane and plasma membrane to reach the periplasm. In eukaryotes, the path from the thylakoid lumen to the outside of the plasma membrane, ignoring thylakoid stacking, involves crossing four membranes (archaeplastida), five membranes (dinoflagellates and euglenids) or six membranes (ochrophytes, cryptophytes, haptophytes, chromerids; Raven *et al.* 2020). These membrane barriers increase the $O_2$ concentration in the cytosol and, increasingly with more membranes around the plastid, in the stroma of eukaryotes and the thylakoid lumen.

For isolated cells of *Gloeobacter*, granted the membrane permeability coefficient reasonably used by Kihara *et al.* (2014), the $O_2$ concentration in the cell during the measured rate of light-saturated photosynthesis is 0.025 mmol $m^{-3}$ greater than that in the medium. This value is about 0.1 that of external $O_2$ concentration in *Synechococcus* sp., for which Kihara *et al.* (2014) assume two layers of thylakoids (a total of four thylakoid membranes in the cell radius), and again using the observed rate of photosynthesis, the $O_2$ concentration in the innermost lumen is 0.25 mmol $m^{-3}$ higher than that in the medium. For *Synechocystis*, assuming three layers of thylakoids (a total of six thylakoid membranes; Kihara *et al.* 2014), the highest $O_2$ concentration in the cells photosynthesizing at the measured rate is 0.064 mmol $m^{-3}$, or 2.6 times that in *Gloeobacter*.

## Implications of intracellular $O_2$ concentration in Gloeobacter and other cyanobacteria before and during the global oxidation event

Kihara *et al.* (2014) discuss the significance of this small increase in intracellular $O_2$ concentration in excess of that in the medium for pre-Global Oxidation Event (GOE) times when there were very low $O_2$ concentrations in the context of generation of reactive oxygen species. Kihara *et al.* (2014) consider the effect of $O_2$ concentration on the production of reactive oxygen species from the triplet, excited state of chlorophyll generated in high light by intersystem crossing. They show that negligible reactive oxygen species would be generated by this mechanism in isolated cells of *Gloebacter* at very low external $O_2$ concentrations. This applies to a slightly lesser extent to the modelled cells of *Synechococcus* and *Synechocystis* (Kihara *et al.* 2014). Kihara *et al.* (2014) suggest that mechanisms that remove reactive oxygen species did not evolve until atmospheric $O_2$ concentrations increased during, and especially after, the GOE.

A further outcome of the small increment in internal $O_2$ in *Gloeobacter* concerns catalysis by its Form IB Rubisco (ribulose-1.5-bisphosphate carboxylase-oxygenase). The oxygenase:carboxylase selectivity in the ancestral Form IB Rubisco is predicted to have been relatively low and similar to that in the extant *Synechococcus* enzyme (Shih *et al.* 2016). With the much higher than present atmospheric $CO_2$:$O_2$ ratio, there would have been little Rubisco oxygenase activity if the $CO_2$:$O_2$ ratio at the Rubisco site was the same as that in air-equilibrated solution. If Rubisco is free in the cytosol, the decreased $CO_2$:$O_2$ at the active site with a diffusive influx of $CO_2$ and efflux of $O_2$ would cause a very limited increase in oxygenase relative to carboxylase activity (Kihara *et al.* 2014; Raven & Beardall 2016; Raven *et al.* 2017). However, the basal, extant cyanobacterium *Gloeobacter* has β-carboxysomes and plasma membrane-located inorganic C transporters ($HCO_3^-$) and channels ($CO_2$) that together constitute a $CO_2$ concentrating mechanism (Rae *et al.* 2013; Mahinthichaichan *et al.* 2018; Sutter *et al.* 2019). Raven *et al.* (2017) discuss the possible timing of origin of the β-cyanobacterial carbon dioxide-concentrating mechanism, having computed that $CO_2$- concentrating mechanisms are required by cyanobacteria when the $CO_2$ concentration is less than 12 times the present atmospheric value. These low $CO_2$ concentrations were probably reached in the Palaeoproterozoic Huronian glaciation and the Neoproterozoic Sturtian and Marinoan glaciations (Raven *et al.* 2017). These low concentrations are despite the requirement for a greater greenhouse effect, with $CO_2$ as a major contributor, in early than in later glaciations as a result of the faint young sun (Raven *et al.* 2017). However, there is the problem of how $CO_2$ concentrating mechanisms could be maintained in the probably higher $CO_2$ conditions of the interglacial between the Palaeoproterozoic and Neoproterozoic glaciations (Raven *et al.* 2017).

It should be emphasised that 'Gloeo' in the generic name *Gloeobacter* refers to its sticky polysaccharide envelope, allowing the natural occurrence of organism in mats on rocks (Saw *et al.* 2013; Mareš *et al.* 2013a). Life in mats means the intracellular $O_2$ excess over that in the medium during photosynthesis is greater than that calculated for isolated *Gloeobacter* by Kihara *et al.* (2014), and may be greater than the calculated values (1300 mmol $m^{-3}$) for *Synechococcus* aggregates of 40 μm radius (Kihara *et al.* 2014). As indicated above, aggregations of *Gloeobacter violaceus* up to 3 mm in diameter also occur in solution cultures (Selstam & Campbell 1996; Sicora *et al.* 2008; Mimuro *et al.* 2011); *Gloeobacter kilaueensis* also forms aggregates in solution culture (Saw *et al.* 2013).

## Synthesis, repair and operation of the photosynthetic apparatus of Gloeobacter in relation to its low-salinity habitat

In *Gloeobacter*, the photosynthetic photochemical reactions, associated redox, and $H^+$ pumping reactions occur in the cell membrane (i.e. inner membrane or plasma membrane) rather than in thylakoids (Rexroth *et al.* 2011). As mentioned above, the cell membrane contains the membrane-associated reactions of respiration and $H^+$ pumping rhodopsin as well as the entry of solutes required for cell growth (Rexroth *et al.* 2011). The oxidizing side of photosystem II, with the $Mg_4CaO_5$ oxygen-evolving complex (OEC), abuts on the periplasm and, via the outer membrane, the external medium (a P phase, in the sense of Mitchell: see Nicholls & Ferguson 2013). Despite the location of the normally thylakoid-located photosynthetic catalysts in the plasma membrane, the photosynthetic reactions of *Gloeobacter violaceus* are similar in most respects to those of other cyanobacteria (Koenig & Schmid 1995; Mulkidjanian *et al.* 2006; Sicora *et al.* 2008; Koyama *et al.* 2008; Bernát *et al.* 2012; Mulo *et al.* 2012; Cardona 2015, 2016). Particularly important in the current context is the similarity of the response to photoinhibition by photosynthetically active radiation to that in other cyanobacteria (Sicora *et al.* 2008; Koyama *et al.* 2008; Bernát *et al.* 2012; Mulo *et al.* 2012; Cardona 2015, 2016).

Raven (2020) points out that, if the $Cl^-$ concentration needed for assembly of the $Mn_4CaO_5$ oxygen-evolving complex is the same as that needed by PSII of isolated *Spinacia* (Vinyard *et al.* 2019), the rate of assembly would be very significantly restricted when the growth medium for *Gloeobacter* was BG11; Sicora *et al.* (2008) found about 30% recovery from photoinhibition by photosyntheticaly active radiation in 2 hours in BG11 medium. Vinyard *et al.* (2019) provide information on the concentration of Mn and Ca needed for the maximum rate of OEC assembly in PSII in *Spinacia*, and these are compared below with the concentrations of Mn and Ca in BG11. What follows is an extension of the analysis of Raven (2020) on the impact of the location of the OEC in *Gloeobacter* on the periplasmic side of the plasma membrane on the function, assembly and reassembly of the OEC from the ion concentrations in BG11. The analysis below takes into account any modification of the $Mn^{2+}$, $Ca^{2+}$ and $Cl^-$ concentrations within the periplasm relative to those in BG11 and ends with possible implications for the maximum specific growth rate of *Gloeobacter*.

Recipes for BG11 vary slightly; according to Andersen *et al.* (2005), BG11 contains 0.508 mol $m^{-3}$ $Cl^-$, 0.324 mol $m^{-3}$ $Ca^{2+}$, and 0.914 mmol $m^{-3}$ $Mn^{2+}$. As a gram-negative bacterium, the cell membrane (i.e. plasma membrane) of *Gloeobacter* is separated from the growth medium by periplasm-containing peptidoglycan polymers, which bear a net negative charge (balanced by diffusible cations, Raven 2020). At the outer surface of the periplasm is the outer membrane with proteinaceous pores that differ from the porins in many other gram-negative bacteria and are generally considered to be non-selectively permeable to inorganic ions, but with no energized transporters moving solutes against the electrochemical potential difference (Kowata *et al.* 2017; Eisenhut 2019). Most cyanobacteria, and in particular, organisms like *Gloeobacter* living epilithically, also have a sheath of polysaccharide, protein and covalently bound negatively charged components such as gluconate, phosphate and sulphate (Schneider & Jürgens 1991). The Donnan effect of the non-diffusible anionic polymers (Briggs *et al.* 1961) means that the diffusible $Cl^-$ concentration in the sheath and periplasm is slightly less than that in BG11, while the diffusible $Ca^{2+}$ and $Mn^{2+}$ concentrations are substantially larger than those in BG11, although their electrochemical potential is the same as that in BG11. In a non-cyanobacterial gram-negative bacterium, the periplasmic-free $Ca^{2+}$ concentration can be accounted for by the Donnan effect (Jones *et al.* 2002).

While there is evidence of Mn accumulation in the periplasm of the freshwater cyanobacterium *Synechocystis* (Keren *et al.* 2002; Stengel *et al.* 2012), this is bound rather than free, and not immediately available for assembly of the OEC. Keren *et al.* (2002) measured $Mn^{2+}$ in the freshwater *Synechocystis* PCC 6803 growing on BG11 medium, showing that there are $1.5 \cdot 10^6$ Mn atoms per cell in the cytosol and thylakoids and 1 $10^8$ Mn atoms per cell in the periplasm. The periplasmic Mn is six co-ordinated $Mn^{2+}$ in a distorted environment, and is released by EDTA treatment, or by the uncoupler CCCP (carbonyl cyanide m-chlorophenyl hydrazine) that dissipates the proton motive force (PMF: electrochemical potential difference) across the plasma membrane as well as the thylakoids. While the mechanism of $Mn^{2+}$ accumulation is not fully understood, it requires a PMF across the plasma membrane, and the great majority of accumulated Mn in the periplasm is not present as free dissolved $Mn^{2+}$. Periplasmic $Mn^{2+}$ accumulation resembles that of inorganic phosphate in the periplasm of marine *Synechococcus* spp. (strains WH 7803, WH 8102, and WH 8102) that is also dependent on the PMF across the plasma membrane (Kamennaya *et al.* 2020).

Comparing these values with those needed for the reassembly of the oxygen-evolving complex in *Spinacia oleracea* Linnaeus, Vinyard *et al.* (2019) found half saturation of $Cl^-$ of 15 mol $m^{-3}$, and saturation at 40 mol $m^{-3}$. In other words, saturation requires more than 70 times the $Cl^-$ concentration in BG11 for the PSII particles containing psbO (see Raven 2020). For $Ca^{2+}$, Vinyard *et al.* (2019) found an optimal (saturating) concentration of 40 mol $m^{-3}$ for OEC reassembly, i.e. more than 100 times the concentration (0.508 mol $m^{-3}$) in BG11. For $Mn^{2+}$, Vinyard *et al.* (2019) found an optimal (saturating) concentration of 0.16 mol $m^{-3}$ for oxygen evolution complex reassembly in *Spinacia*; this is well over 100 times the concentration (0.914 mol $m^{-3}$) in BG11. Of course, there is a large phylogenetic gap between *Gloeobacter* and *Spinacia*, and there are differences in the *P*-side subunits of PSII (Thornton *et al.* 2004), but the differences in the saturating concentrations of $Cl^-$, $Ca^{2+}$ and $Mn^{2+}$ for oxygen-evolving complex reassembly in *Spinacia* and the concentrations in BG11 are over one ($Cl^-$) and over two ($Ca^{2+}$, $Mn^{2+}$) orders of magnitude. These differences are consistent with slower repair of photoinhibitory damage to PSII and the OEC in *Gloeobacter* relative to other oxygenic photosynthetic organisms. Whether this causes slower repair of photodamage, and even causes slower growth, in *Gloeobacter* requires further study. Regardless, the synthesis (and re-synthesis) of photosynthetic membrane protein complexes occurs in the 'orange' areas of the plasmalemma, while they function in the 'green' areas (Rexroth *et al.* 2011; Nickelsen & Rengstl 2013).

In all known cyanobacteria other than *Gloeobacter*, photosynthesis occurs in thylakoids, and the concentration of $Cl^-$, $Ca^{2+}$, and $Mn^{2+}$ in the thylakoid lumen, the P phase containing the OEC, could be increased by the occurrence of energized transport at the cell membrane and/or thylakoid membrane. Eisenhut (2019) reviews evidence on Mn transport in cyanobacteria. Ritchie (1992) provides data on active $Cl^-$ influx at the cell membrane in *Synechococcus leopoliensis* (Raciborski) Komárek PCC 7942, yielding internal mean concentrations (cytosol plus thylakoid lumen) of 17.2 ± 0.85 mol $m^{-3}$ in the light and 1.24 ± 0.11 mol $m^{-3}$ in the dark in PB11 medium. While the distribution of $Cl^-$ between the cytosol and the thylakoid lumen is not known, Herdean *et al.* (2016) show that a voltage-gated $Cl^-$ channel occurs in the cyanobacterial thylakoid membrane, and presumably functions in the parsing of the $H^+$ electrochemical potential difference across the thylakoid membrane into electrical potential difference and pH difference components (Raven 2020). As Raven (2020) points out, this is unlikely to occur in the plasma membrane of *Gloeobacter* where $Cl^-$ is probably not at electrochemical equilibrium. Chechetto *et al.* (2012) show that a thylakoid $K^+$ channel in cyanobacteria has a similar role parsing the $H^+$ electrochemical potential difference. Ritchie (1991) shows that the mean intracellular $K^+$ in

BG11 (0.35 mol $K^+$ $m^{-3}$) for *Synechococcus leopoliensis* PCC 7942 is about 100 mol $m^{-3}$, adequate for parsing of the $H^+$ electrochemical potential difference across the thylakoid membrane using a thylakoid membrane $K^+$ channel. This role has not been tested in *Gloeobacter*.

In addition to these requirements for the assembly of the OEC, in the case of $Cl^-$ there is also a requirement for the functioning of photosystem II (Raven 2017, 2020). There have been a few contrary reports; a recent report is of the lack of the $Cl^-$ requirement resulting from mutation of a putative $Cl^-$ efflux transporter in a mutant of *Synechocystis* sp. 6803 deficient in cytochrome C550 (Kobayashi *et al.* 2006). This finding has been neither independently replicated nor refuted; however, the majority view is that $Cl^-$ is essential for oxygenic photosynthesis functioning as well as for assembly and reassembly of the OEC. Data from vascular plants show a requirement for 2 mol $m^{-3}$ dissolved $Cl^-$ to saturate the two binding sites for $Cl^-$ in photosystem II (Raven 2020). If this is the case for *Gloeobacter*, then the photosynthetic requirement for $Cl^-$ would not be saturated in BG11 medium. Another role for $Cl^-$ in photosynthesis by organisms with thylakoids is, with $K^+$, in parsing the $H^+$ electrochemical potential difference across the thylakoid membrane using selective ion channels (see previous paragraph). For *Gloeobacter*, any such parsing role of ion channels in the plasmalemma must be compatible with the roles of $K^+$ and $Cl^-$ in osmoregulation and (especially for $K^+$) enzyme activation; unfortunately, little is known about these functions in *Gloeobacter*. It is known that the $CF_0CF_1$ ATP synthase of *Gloeobacter violaceus* has 15 c-subunits, so the $H^+$ flux needed to phosphorylate 1 mole of ATP based on structural biology, is 15/3 or 5 moles $H^+$. This stoichiometry is found in some thylakoid-containing cyanobacteria (Pogoryelov *et al.* 2007), and means that, for a given free energy of ATP hydrolysis in vivo, a smaller $H^+$ electrochemical potential difference is required for ATP synthesis than for cyanobacteria with 14, or especially, 13, c-subunits. In *Gloeobacter violaceus*, the ATP synthase stoichiometry may be related to the occurrence of all membrane-associated bioenergetic processes in the cell membrane. Davis & Kramer (2020) suggest that the higher number of c-subunits in ATP synthases in photosynthetic membranes than in respiratory membranes is related to the use of a large pH difference component of a PMF). This seems more plausible for thylakoid membranes, when the P (lumen) side of the membrane has a relatively unconstrained pH, at least compared to the P (periplasmic) side of the membrane plasma membrane of *Gloeobacter*. Measurements of the pH difference across the *Gloeobacter* plasma membrane over a range of external medium pH values show that the internal (cytosol) pH varies with external pH more than in other cyanobacteria, and other photosynthetic organisms (Belkin *et al.* 1987; Hinterstoissen & Peschek 1987). There are no measurements of the electrical component of the PMF at the *Gloeobacter* plasma membrane. For the *Gloeobacter* proton-pumping rhodopsin, expressed in *Xenopus* oocytes, the maximum PMF that can be generated is 260 mV (no pH difference

**Table 2.** Specific growth rate of the thylakoid-less Cyanobacteria *Gloeobacter*, thylakoid-containing Cyanobacteria, and the anoxygenic photosynthetic green bacterium *Chlorobium*. Data are for organisms growing under conditions yielding the maximum growth found in the cited publication. Since a range of temperatures were used, as well as the growth rate at the experimental temperature, the growth rates have been normalized to 25°C assuming a $Q_{10}$ of 2.

| Organism | Temperature/ °C | Photon flux density/ μmol $m^{-2}$ $s^{-1}$ | μ/ $d^{-1}$ | μ/ $d^{-1}$ normalized to 25°C | Reference |
|---|---|---|---|---|---|
| *Gloeobacter violaceus* PCC 7421 | 26 | 50 | 0.0115 | 0.0107 | Nguyen *et al.* (2012) |
| *Gloeobacter violaceus* PCC 7421 | 24 | 20 | 0.016 | 0.017 | Herrmann & Gehringer (2019) |
| *Synechocystis* PCC 6803 | 28 | 500 | 0.11 | 0.089 | Jahn *et al.* (2018) |
| *Synechococcus elongatus* (Nägeli) Nägeli UTEX 2993 | 41 | 500 | 0.36 | 0.118 | Yu *et al.* (2015) |
| *Synechococcus* PCC 7942 | 38 | 300 | 0.17 | 0.069 | Yu *et al.* (2015) |
| *Synechococcus* PCC 7942 | 33 | 120 | 0.052 | 0.030 | Kuan *et al.* (2015) |
| *Synechococcus* PCC 8101 | 25 | 75 | 0.067 | 0.067 | Binder & Liu (1998) |
| *Chlorobium limicola* forma *thiosulphatophilum* Nadson ATCC 17092 | 30 | 160 | 0.042 | 0.030 | Cork *et al.* (1983) |
| *Chlorobium tepidum* Wahlund, Woese, Castenholz & Michigan WI 2321 | 47 | 170 | 0.30 | 0.045 | Frigaard *et al.* (2002) |

component), equivalent to 25 kJ per mole photons driving force for downhill proton re-entry (Vogt *et al.* 2013). Parsing the PMF into electrical potential difference and pH difference components is important for several photosynthetic processes (Davis *et al.* 2016).

Based on metagenomic data, the closest living relative of *Gloeobacter* spp, *candidatus Aurora vandensis*, has oxygenic photosynthesis with binding sites for $Mn^{2+}$, $Ca^{2+}$ and $Cl^{-}$ forming an oxygen-evolving complex; however, it is not clear whether *candidatus Aurora vandensis* has thylakoids (Grettenberger *et al.* 2020), and/or whether the assembly of the oxygen-evolving complex involves interaction with external (to the cytoplasmic membrane) $Mn^{2+}$, $Ca^{2+}$, and $Cl^{-}$.

## OUTCOME OF STRUCTURE AND METABOLISM OF *GLOEOBACTER* FOR RESOURCE-SATURATED SPECIFIC GROWTH RATE

Table 2 gives specific growth rate values derived from the literature of: (1) *Gloeobacter*, (2) other unicells with thylakoids (as do all non-*Gloeobacter* cyanobacteria), and (3) *Chlorobium* Nadson 1906, an anoxygenic green sulphur-oxidizing and photoferrotrophic bacterium that, like *Gloeobacter*, has photochemistry located in the plasma membrane. Since the growth rate data were obtained at a variety of temperatures, and tested at or near the highest growth rate, the data are normalized to a growth temperature of 25°C, assuming a $Q_{10}$ of 2. The normalized values show that *Gloeobacter* grows less rapidly than either the other cyanobacteria or the *Chlorobium* species. The meta-analysis of Nielsen (2006) of the specific growth rate of 22 species of unicellular and colonial cyanobacteria as a function of cell or colony size shows an approximately 50-fold range of growth rates for cyanobacteria of a size similar to that of *Gloeobacter*, with *Gloeobacter* among the slowest growers. Nielsen (2006) did not adjust rates for differences in growth temperatures. Flynn (2009) considers the range of resource-saturated specific growth rates among photoautotrophic micro-organisms to be resource diversions that limit growth rate but improve evolutionary fitness, e.g. anti-grazer defences.

The comparison of specific growth rates among cyanobacteria is consistent with a lower maximum net photosynthetic rate in *Gloeobacter*. This results from the smaller volume-specific area for the membrane-associated processes of photosynthesis in *Gloeobacter* with the absence of thylakoids, and hence the location of photosynthetic and respiratory catalysis as well as nutrient transporters, ion-regulating channels and transporters, and $H^{+}$-pumping rhodopsin, in the plasma membrane, as well as constraints on synthesis, repair and function of the periplasm side of photosystem II resulting from growth in fresh water. This conclusion about the membrane area per cell available for photosynthetic reactions in *Gloeobacter* depends on a similar size of thylakoid-containing species to that of *Gloeobacter*; a significantly lower size of *Gloeobacter* would attenuate the photosynthetic area per unit cell volume effect. The light-saturated rate of gross photosynthesis, assuming that respiration continues in the light at the same rate as in the dark, in *Gloeobacter violaceus*, on a chlorophyll *a* basis (57 mmol $O_2$ $mol^{-1}$ chlorophyll *a* $s^{-1}$) is almost as high as that of *Synechocystis* (63 mmol $O_2$ $mol^{-1}$ chlorophyll *a* $s^{-1}$) measured under the same conditions. However, the much higher rate of dark respiration in *Gloeobacter* (25 mmol $O_2$ $mol^{-1}$ chlorophyll *a* $s^{-1}$) than in *Synechocystis* (1.2 mmol $O_2$ $mol^{-1}$ chlorophyll *a* $s^{-1}$) means a large difference in net photosynthesis (*Gloeobacter* with 32 mmol $O_2$ $mol^{-1}$ chlorophyll *a* $s^{-1}$; *Synechocystis* with 61 mmol $O_2$ $mol^{-1}$ chlorophyll *a* $s^{-1}$). Of course, relating the net photosynthesis to the potential for net C assimilation and potential growth rate requires a per cell or per cell C chlorophyll *a* content, which is not available for *Gloeobacter*. Importantly, all of the thylakoid-containing cyanobacteria in Table 2 are planktonic, while the epilithic habitat of *Gloeobacter* involves a larger relative production of extracellular polysaccharides, imposing a diversion of more of the organic C produced in photosynthesis from growth of the cell within the outer membrane.

The comparison of *Gloeobacter* with *Chlorobium* requires further analysis. *Chlorobium* has no proton-pumping rhodopsins, and only one photochemical reaction in photosynthesis. It is also likely that the light-harvesting chlorosomes on the cytosol side of the plasma membrane of *Chlorobium* spare the occurrence of light-harvesting pigments in the membrane to a greater extent than phycobilisomes in *Gloeobacter*. Hauska *et al.* (2001) give values of 200,000 bacteriochlorophyll *c* (or *d* or *e*) per chlorosome, and 40 reaction centres in the cell (plasma) membrane per chlorosome, i.e. 5000 bacteriochlorophyll per reaction centre. Koyama *et al.* (2008) found 1 PSII reaction centre per 185 chlorophylls in *Gloeobacter violaceus*, and 1 PSII reaction centre per 330 chlorophylls in *Synechocystis*. There seem to be no data on the phycobilin: chlorophyll ratio in *Gloeobacter*; the purple colour of this organism (Mareš *et al.* 2013a) is not necessarily all due to phycobilins in view of the presence of ion-pumping rhodopsin (Rexroth *et al.* 2011). In other cyanobacteria, the ratio of mol C-phycocyanin chromopores (phycocyanobilin):mol chlorophyll *a* is 0.16 for *Oscillatoria agardhii* Gomont (Post *et al.* 1986), and the ratio of mol C phycoerythrin chromopores (phycoerthinobilin):mol C phycocyanin chromophores (phycocyanobilin):mol chlorophyll *a* is 4.2:0.13 in *Pseudoanabaena* sp. (Mishra *et al.* 2012).

How does the low maximum specific growth rate of *Gloeobacter* relate to its ecology? There are no estimates of growth rate of *Gloeobacter* in its natural environment, but 1–5 mm thick mats of the phylogenetically derived freshwater and terrestrial cyanobacterial mats of *Nostoc commune* Vaucher ex Bornet & Fahault have doubling times of not less than 10–14 days, i.e. a specific growth rate of 0.05–0.07 $d^{-1}$ (Sand-Jensen 2014). The light-saturated rate of photosynthesis on an area basis of fully hydrated mats of *Nostoc commune* is at the lower end of the range for planar or cylindrical macrophytic algae (Sand-Jensen 2014; Raven *et al.* 2019), and related to the very high photosynthetic pigment per unit area and hence the large package effect (self-shading) of *Nostoc commune* mats, the mol $O_2$ produced per mol absorbed photon is low (Raven *et al.* 2014; Sand-Jensen 2014). The photosynthesis:respiration ratio in mats of *Nostoc commune* is low at 2.5, and the light compensation value is high at 9.5 μmol photon $m^{-2}$ $s^{-1}$, again apparently a result of

self-shading and high $O_2$ and low $CO_2$ in the mats (Sand-Jensen 2014). The low photosynthesis:respiration ratio and specific growth rate, and high light compensation point, of *Gloeobacter violaceus* relative to *Synechococcus* during growth in liquid culture may be partly related to the formation of cell aggregates in liquid culture of *Gloeobacter* species (Selstam & Campbell 1996; Sicora *et al.* 2008; Mimuro *et al.* 2011; Saw *et al.* 2013). This aggregation effect might be exacerbated by growth of *Gloeobacter violaceus* in mats, especially if the mats are as thick as for *Gloeobacter kilauensis* (5 mm; Saw *et al.* 2013). It seems likely that *Gloeobacter* would grow more slowly than mats of *Nostoc commune* of a comparable thickness and under otherwise similar conditions.

## UV AS A CONSTRAINT ON PHOTOSYNTHESIS BEFORE THE GOE

A potential constraint on photosynthesis before the Global Oxidation Event (GOE) was the greater UV flux, and especially UVC (< 280 nm), in the absence of stratospheric ozone as a UV screen (Cockell & Raven 2007; Zerkle *et al.* 2012; Mioszewska *et al.* 2018; Kugler & Dong 2019). Photosynthetically active radiation, i.e. (380)–400–700 (–730) nm is required for oxygenic photosynthesis, so any screening of UV must still allow the photosynthetic light-harvesting pigments to be supplied with photosynthetically active radiation (Cockell & Raven 2007; Zerkle *et al.* 2012; Mioszewska *et al.* 2018; Kugler & Dong 2019; Garcia-Pichel *et al.* 2019). A possible global screening mechanism is a UV-absorbing organic atmospheric haze with lifetime of individual molecules increased by the absence of oxidation by atmospheric $O_2$ (Zerkle *et al.* 2012). Marine, and probably freshwater ecosystems, would have had dissolved $Fe^{2+}$ as a UV screen before oxidation by $O_2$ to $Fe^{2+}$, although some $Fe^{2+}$ would be removed by anoxygenic photosynthesis using $Fe^{2+}$ as electron donor. On land and in marine and freshwater microbial mats, phyllosilicates (Fe + Si) could act as a local UV refuge for cyanobacteria with UV absorbed to a greater extent than photosynthetically active radiation (Mioszewska *et al.* 2018; Kugler & Dong 2019). This is a special case of endo- or hypolithic UV refuges (Cockell & Raven 2007). The stromatolite-forming marine cyanobacterium *Geitlerionema* is very UV-C resistant (Popall *et al.* 2020). Cockell *et al.* (2009) have estimated the potential for net $O_2$ production by endo- and hypo-lithic oxygenic terrestrial photolithotrophs covering the land surface; the upper limit, assuming essentially all the organic carbon produced escapes oxidation, would produce the present atmospheric $O_2$ mass in *c.* 1 Ma. The limited organic C oxidation would deplete atmospheric $CO_2$; estimates of neo-Archaean $CO_2$ partial pressure have been are as high as 70 kPa (Lehmer *et al.* 2020), compared to 41 Pa today, although $CO_2$-concentrating mechanisms are apparently an ancestral cyanobacterial trait (Raven *et al.* 2017). Resupply of $CO_2$ from vulcanism would also involve efflux of reducing gases and lava that consume $O_2$.

Finally, the UV flux incident on cyanobacterial cytoplasm can be decreased by extracellular, UV-absorbing compounds synthesized by the cells. One such compound is scytonemin, produced by many later-evolving cyanobacteria (not *Gloeobacter*) that screen UVA whose damaging action is enhanced by $O_2$ (Garcia-Pichel *et al.* 2019). The common intracellular UV-screening mycosporine-like amino acids have not been reported in *Gloeobacter* (D'Agostino *et al.* 2019).

## COULD CYANOBACTERIA ON LAND AND/OR IN FRESH WATER HAVE ACCOUNTED FOR ORGANIC C BURIAL AND CORRESPONDING $O_2$ ACCUMULATION DURING THE GOE?

Granted a terrestrial or freshwater origin of cyanobacteria, the sequence of habitats colonized by cyanobacteria is terrestrial and/or freshwater, then marine benthos, and finally marine plankton (Blank & Sánchez-Baracaldo 2010; Blank 2013a; Sánchez-Baracaldo *et al.* 2014; Lyons *et al.* 2014; Sánchez-Baracaldo 2015; Dick *et al.* 2018; Schad *et al.* 2019; Sánchez-Baracaldo *et al.* 2019; Sánchez-Baracaldo & Cardona 2020). In considering whether terrestrial and/or freshwater cyanobacteria could have been responsible for the GOE 2.3–2.4 Ga ago, a necessary preliminary is that terrestrial and/or freshwater cyanobacteria existed prior to the GOE. Direct fossil evidence for Archaean cyanobacteria is lacking (Demoulin *et al.* 2019). However, evidence consistent with an early Archaean (*c.* 3.2 Gya) origin of photosystem II, the version of Type II photosynthetic reaction centres that, with the oxygen-evolving complex, is the only biological mechanism that generates $O_2$ from $H_2O$ (Cardona 2019; Cardona *et al.* 2019; Tamura *et al.* 2020). This does not necessarily mean that there were cyanobacteria as currently known, and multigene molecular phylogenetic data suggest the origin of the crown group cyanobacteria (genome of extant *Gloeobacter*) at 2.6–-2.7 Gya (Sánchez-Baracaldo *et al.* 2014; Sánchez-Baracaldo *et al.* 2017a, 2017b; Dick *et al.* 2018; Cardona *et al.* 2019; Hamilton 2019; Sánchez-Baracaldo & Cardona 2020). The molecular phylogenies suggest that a limited number of early branching cyanobacteria taxa survived the GOE, with a predominance of freshwater and/or terrestrial species (Sánchez-Baracaldo *et al.* 2014; Sánchez-Baracaldo *et al.* 2017a, 2017b; Dick *et al.* 2018; Cardona *et al.* 2019; Grettenberger *et al.* 2020; Sánchez-Baracaldo & Cardona 2020). In other words, only a handful of basal taxa left descendants, since most of the modern cyanobacteria groups diversified after the GOE (Sánchez-Baracaldo & Cardona 2020).

Were the freshwater and terrestrial biospheres adequate to account for the GOE (Sánchez-Baracaldo 2015)? The accumulation of $O_2$ in the biosphere requires organic C burial in excess of that stoichiometric with $O_2$ accumulation. This is because some of the $O_2$ produced is consumed in oxidation of $Fe^{2+}$ and $S^{2-}$ on the land surface, in the surface of water bodies and by reducing gases in the atmosphere (Lyons *et al.* 2014). There was emergent continental crust from *c.* 3.5 Ga ago (Buick *et al.* 1995), microbially-induced sedimentary structures from a *c.* 3.38 Gya coastal marine environment (Noffke *et al.* 2013), palaeosols from *c.* 3 Ga ago (Retallak *et al.* 2016) and lakes with stromatolites from *c.* 2.7 Ga ago (Buick 1992; Awramik & Buhheim 2009; Stueken *et al.* 2017; Willmeth *et al.* 2019). Willmeth *et al.* (2019) suggest from the occurrence of fenestrae in the stromatolites could be related to

oxygenic photosynthesis. Lakes with benthic (and planktonic) cyanobacteria illustrated by Dick *et al.* (2018, fig. 2a) depict pre-GOE biogeochemistry. Sumner *et al.* (2015) suggest that microbial mats in Lake Fryxel (Antarctica) are an analogue of Archaean oxygen oases.

A major quantitative role of freshwater cyanobacteria in the GOE requires burial of photosynthetically produced organic C in stoichiometric excess of $O_2$ accumulation to accommodate inorganic $O_2$ consumption in oxidation of $Fe^{2+}$, $S^{2-}$, and reducing gases. Cyanobacteria in today's freshwater habitats have a substantial role in organic C burial (about 0.013 Pmol C per year), which, other things being equal, equals 0.013 Pmol $O_2$ released (Mendonça *et al.* 2017). However, there are large organic C inputs to fresh waters from terrestrial productivity, the metabolism of which means that 0.027 Pmol $CO_2$ is released from fresh waters, i.e. about 0.027 mole $O_2$ uptake (Mendonça *et al.* 2017). The same applies to combined N input from the catchment to freshwater habitats, with net $N_2O$ and $N_2$ losses to the atmosphere showing that denitrification and anammox reactions exceed $N_2$ fixation, mainly by cyanobacteria (Loeks-Johnson & Cotner 2020). Organic C burial in fresh waters at the time of the GOE would have had a much smaller input from catchment primary productivity.

There is fossil evidence of Archaean (*c.* 3.2–2.6 Ga) terrestrial microbial mats with organic carbon isotope natural abundance values consistent with a photosynthetic origin (Watanabe *et al.* 2000; Lenton & Daines 2017; Homann *et al.* 2018; Thomazo *et al.* 2020). Finke *et al.* (2019) and Thomazo *et al.* (2020) suggest that extant cyanobacteria-dominated soil crusts are modern analogues of the Archaean continental biosphere, using C and N isotope natural abundance data from recent and *c.* 3.2-billion-year-old crusts; natural abundance of N isotopes is more liable to diagenetic change than C isotopes (Handley & Raven 1992). Elbert *et al.* (2012) show that today's cryptogamic covers, with a significant contribution from cyanobacteria, contribute about 7% to terrestrial primary productivity, with vascular plants providing habitats (as phorophytes, and by shading). Edwards *et al.* (2015) and Raven (2018) discuss the role of cyanobacteria and other components of cryptogamic cover before the evolution of vascular plants. The high UVB and, especially, UVC flux incident on the Earth's surface before the development of a stratospheric ozone shield might mean that both terrestrial and freshwater oxygenic photosynthesis are endolithic and hypolithic, with rocks intercepting UVB and UVC relative to photosynthetically active radiation. Cockell *et al.* (2009) discuss the possible capacity for net $O_2$ production in endo- and hypolithic habitats. UV flux problems with Archaean photosynthesis are further discussed below.

Molecular phylogenetic studies render the presence of modern benthic marine cyanobacteria at the time of the GOE rather unlikely (Sánchez-Baracaldo *et al.* 2014; Sánchez-Baracaldo *et al.* 2017a, 2017b; Dick *et al.* 2018; Cardona *et al.* 2019; Grettenberger *et al.* 2020; Sánchez-Baracaldo & Cardona 2020). However, ancestral stem groups of marine mat-forming cyanobacteria were becoming established around the time of the GOE (Blank & Sánchez-Baracaldo 2010). The evidence of Neoarchean photosynthesis in a coastal lagoon (Eroglu *et al.* 2017) does not necessarily mean oxygenic photosynthesis (but see Koehler *et al.* 2018; Cardona 2019; Ossa *et al.* 2019). The natural abundance stable isotope ratio of carbonate C and organic C (Eroglu *et al.* 2017) are just at the limit of the range of extant cyanobacteria using Form IB Rubisco (McNevin *et al.* 2007), but also at the limit of the range of photosynthetic proteobacteria using Form II Rubisco (McNevin *et al.* 2007) or Form IC Rubisco (Thomas *et al.* 2018). However, the isotopic data (Eroglu *et al.* 2017) are also consistent with autotrophic $CO_2$ assimilation, using phosphoenolpyruvate carboxykinase as the initial carboxylase in a $C_4$ pathway such as is proposed for the marine ulvophycean green alga *Udotea flabellum* (J. Ellis & Solander) M. Howe (Raven *et al.* 2002).

The extent of terrestrial habitats might not be adequate to explain the GOE (Sánchez-Baracaldo *et al.* 2014; Sánchez-Baracaldo 2015; see Lyons *et al.* 2014) although terrestrial cyanobacteria play important subsequent roles in bioengineering the land surface of our planet today (Edwards *et al.* 2015; Raven 2018). Based on ancestral state reconstruction, it is likely that there were marine (non-planktonic) cyanobacteria (Blank & Sánchez-Baracaldo 2010), and multicellular/filamentous cyanobacteria (Schirrmeister *et al.* 2013, 2016) at the time of the GOE (see also Sánchez-Baracaldo *et al.* 2014; Sánchez-Baracaldo 2015). Furthermore, the GOE occurred at about the time that the modern PSII configuration evolved (Cardona *et al.* 2019). Even if there were benthic marine cyanobacterial mats by the time of the GOE (Sánchez-Baracaldo *et al.* 2014; Sánchez-Baracaldo 2015), there could be limits on the extent of their net primary productivity, and hence on their contribution of organic C burial and $O_2$ production to the GOE (Dick *et al.* 2018).

## CYANOBACTERIA AND THE LOMAGUNDI-JATULI EVENT

The Lomagundi–Jatuli Event (LJE) is recognized by an increase of up to 10‰ in the $\delta^{13}C$ of marine carbonates 2.2–2.1 Gya. This is interpreted as a large increase in photosynthetic primary productivity, preferentially removing the $^{12}C$ from the inorganic C pool relative to $^{13}C$, leaving a relatively $^{13}C$-enriched (higher $\delta^{13}C$) inorganic C pool in the surface ocean (Paiste *et al.* 2020). If the LJE were ocean-wide it would have involved very large organic C burial and $O_2$ production and would require large primary production rates in the open ocean with the corresponding occurrence of planktonic cyanobacteria. However, the data in Paiste *et al.* (2020) show that the LJE was a coastal ocean phenomenon, with much smaller effects in open ocean carbonate sediments. While this agrees with the absence of planktonic cyanobacteria 2.2–2.1 Gya, the most basal lineages of cyanobacteria indicate that shortly after the diversification of the last common ancestor of cyanobacteria, lineages were colonizing a wide range of habitats. Molecular phylogenetic data and trait evolution analyses of cyanobacteria suggest that marine lineages were already present by around the GOE, but modern mat-forming, salinity-tolerant cyanobacteria evolved after this event

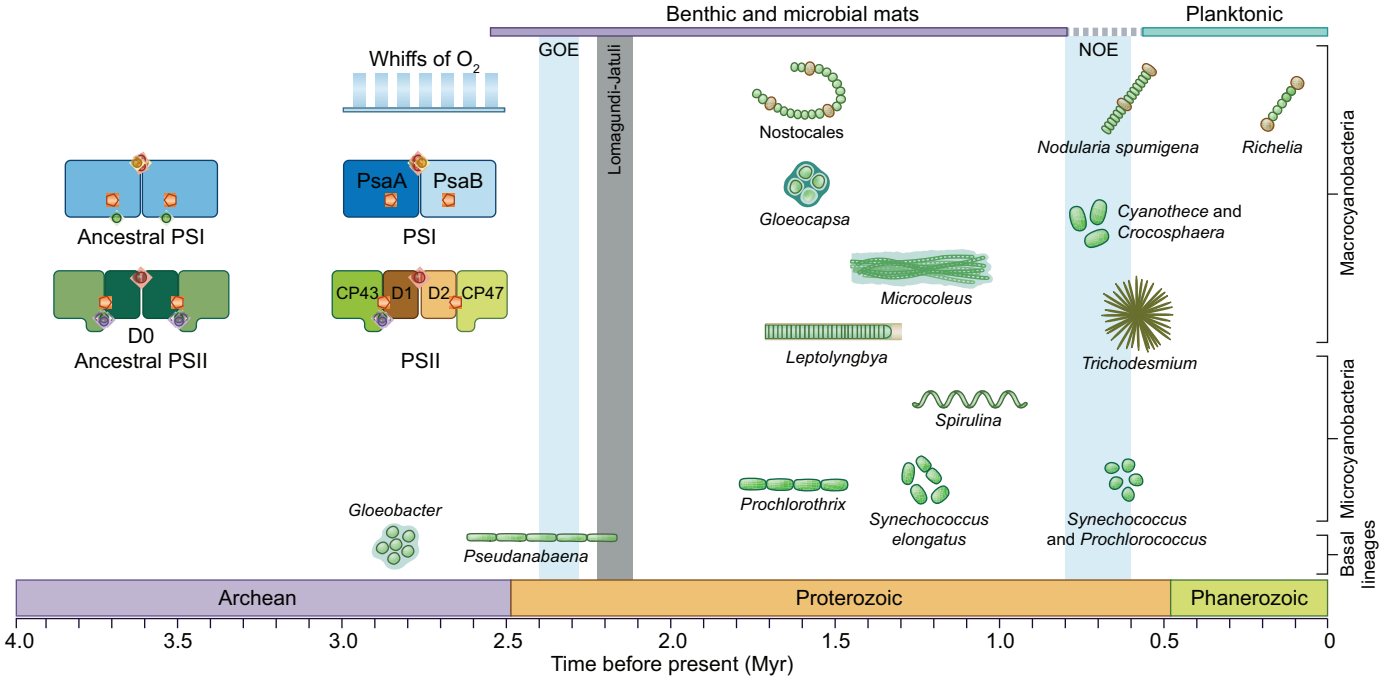



**Fig. 1.** Timing of the emergence of PSI, PSII and some cyanobacterial lineages. Molecular clock age estimates for PSI (Cardona 2018), PSII (Cardona *et al.* 2019), *Gleobacter* (Schirrmeister *et al.* 2015) and major clades and taxa (Sánchez-Baracaldo 2015; Sánchez-Baracaldo *et al.* 2017a, 2017b). The timing of the Great Oxidation Event (GOE; Bekker *et al.* 2004), the Lomagundi-Jatuli Excursion (Kump *et al.* 2011) and Neoproterozoic Oxidation Event (NOE; Och & Shields-Zhou 2012) are illustrated with vertical lines. Ancestral forms of PSII and PSI emerged in the early Archaean or early Proterozoic. D0 refers to an ancestral core subunit before the gene duplication that led to D1 and D2. The Cyanobacteria crown group inherited a heterodimeric photosystem II now shared by all oxygenic phototrophs. The majority of extant Cyanobacteria evolved after the GOE, and planktonic groups diversified towards the end of the Proterozoic and the Cretaceous periods. Cartoons are not drawn to scale. The dominant Cyanobacteria in the Precambrian were benthic, freshwater and marine, and some were terrestrial. Fig. 1 is modified from fig. 3 in Sánchez-Baracaldo & Cardona (2020).

(Blank & Sánchez-Baracaldo 2010; Blank 2013b; Sánchez-Baracaldo *et al.* 2014; Lyons *et al.* 2014; Sánchez-Baracaldo 2015; Dick *et al.* 2018; Sánchez-Baracaldo & Cardona 2020).

## CONCLUSIONS

The basal cyanobacterium *Gloeobacter* lacks thylakoids and grows slowly in liquid culture under what are believed to be conditions allowing maximum growth relative to other cyanobacteria examined. While it is tempting to relate this slow growth to the absence of thylakoids and a low maximum rate of photosynthesis, more data are needed to test this possible relationship. Slow growth in culture is unlikely to be a result of the tendency of cells to aggregate, as occurs in growth in epilithic mats in nature, if the growth conditions are not limited by light or nutrients; aggregation would increase growth limitation by low light (increased package effect in photosynthesis) or nutrients (increased diffusive limitation on supply to the plasma membrane) such as would occur when these resources are limiting. The absence of thylakoids in *Gloeobacter*, and its low-salinity habitat, means that the oxygen-evolving complex of photosystem II on the periplasmic side of the plasma membrane is exposed to concentrations of $Mn^{2+}$, $Ca^{2+}$ and $Cl^-$ that are probably limiting for the rate of synthesis, and re-synthesis after photoinhibition, of the oxygen-evolving complex; this might limit the growth rate. Furthermore, low external $Cl^-$ concentrations could limit photosynthesis even with an assembled oxygen-evolving complex.

*Gloeobacter* has retained basal cyanobacterial traits; could such cyanobacteria living on land or in fresh water have been responsible for the GOE in the Palaeoproterozoic? There is evidence of whiffs of $O_2$, and microbial mats and organic C storage on the continental surface in the Archaean. With the best available dating of the molecular phylogeny of cyanobacteria, it is very unlikely that there were marine planktonic cyanobacteria. Although there is evidence consistent with cyanobacteria on the continental surface having a role in the GOE, more evidence is needed on whether organic C burial can explain the increase in $O_2$. More work is needed to test a continental surface or marine benthic origin of the subsequent Palaeoproterozoic Lomagundi-Jatuli excursion.

## ACKNOWLEDGEMENTS

Discussions with Tony Prave have been very helpful. The comments of two anonymous reviewers, and of David Garbary, have significantly improved the paper. We thank Giorgio Bianchini for assistance with the graphics (Fig. 1).

The University of Dundee is a registered Scottish charity, No. 015096.

We dedicate this paper to the memory of Professor Mario Giordano: scholar, gentleman, colleague and friend.

## FUNDING

Funding support for this work came from a Royal Society University Research Fellowship for PS-B.

## REFERENCES


Andersen R.A., Berges J.A., Harrison P.J. & Watanabe M.M. 2005. Appendix A. Recipes for freshwater and seawater media. In: *Algal culturing techniques* (Ed. by R.A. Andersen), pp 429–538. Elsevier, Phycological Society of America, Amsterdam, Netherlands.

Awramik A. & Buhheim P. 2009. A giant, late Archean lake system: the Meentheena Member (Tubiana Formation, Fortescue Group), Western Australia. *Precambrian Research* 174: 215–240. DOI: 10.1016/j.precamres.2009.07.005.

Bekker, A., Holland, H.D., Wang, P-L, Rumble III, D., Stein, H.J., Hannah, J.L., Coetzee, L.L. & Beukes, N.J. 2004. Dating the rise of atmospheric oxygen. *Nature* 427: 117–120. DOI: 10.1038/nature.02260.

Belkin S., Mehhorn R.J. & Packer L. 1987. Proton gradients in intact cyanobacteria. *Plant Physiology* 84: 25–30. DOI: 10.1104/pp.84.1.25.

Bernát G., Schreiber U., Sendtko E., Stadnichuk I.N., Rexroth S., Rögner M. & Koenig F. 2012. Unique properties vs. common themes: the atypical cyanobacterium *Gloeobacter violaceus* PCC 7421 is capable of state transitions and blue-light-induced fluorescence quenching. *Plant and Cell Physiology* 53: 528–542. DOI: 10.1093/pcp/pcs009.

Binder B.J. & Liu Y.C. 1998. Growth rate regulation of rRNA content of a marine *Synechococcus* (cyanobacteria) strain. *Applied and Environmental Microbiology* 64: 3346–3351. DOI: 10.1128/AEM.64.9.3346-3351.1998.

Blank C.E. 2013a. Phylogenetic distribution of compatible solute synthesis genes support a freshwater origin for cyanobacteria. *Journal of Phycology* 49: 880–895. DOI: 10.1111/jpy.12098.

Blank C.E. 2013b. Origin and early evolution of photosynthetic eukaryotes in freshwater environments – reinterpreting Proterozoic palaeobiology and biogeochemical processes in light of trait evolution. *Journal of Phycology* 80: 1040–1055. DOI: 10.1111/jpy.12111.

Blank C.E. & Sánchez-Baracaldo P. 2010. Timing of morphological and ecological innovations in the cyanobacteria – a key to understanding the rise of atmospheric oxygen. *Geobiology* 8: 1–23. DOI: 10.1111/j.1472-4669.2009.00220.x.

Briggs G.E., Hope A.B. & Robertson R.N. 1961. *Electrolytes and plant cells. Botanical monographs, Volume 1*. Blackwell, Oxford, UK. 217 pp.

Buick R. 1992. The antiquity of oxygenic photosynthesis: evidence from stromatolites in Archaean lakes. *Science* 255: 74–77. DOI: 10.1126/science.11536492.

Buick R., Thornett J.R., McNaughton N.J., Smith J.B., Barley M.E. & Savage M. 1995. Record of emergent continental crust ~ 3.5 billion years ago in the Pilbara craton of Australia. *Nature* 375: 574–577. DOI: 10.1038/375574a0.

Burnap R.L., Hagemann M. & Kaplan A. 2015. Regulation of $CO_2$ concentrating mechanism in cyanobacteria. *Life* 5: 348–371. DOI: 10.3390/life5010348.

Cabello-Yeves P.J. & Rodriguez-Valera F. 2019. Marine-freshwater transitions require extensive changes in the predicted proteome. *Microbiome* 7: Article 117. DOI: 10.1186/s40168-019-0731-5.

Cabello-Yeves P.J., Picazo A., Camacho A., Callieri G., Rosselli R., Rodo-Garcia J.J., Coutinho F.H. & Rodriguez-Valera F. 2018. Ecological and genomic features of two widespread freshwater picocyanobacteria. *Environmental Microbiology* 20: 3757–3771. DOI: 10.1111/1462-2920.14377.

Cardona T. 2015. A fresh look at the evolution and diversification of photochemical reaction centers. *Photosynthesis Research* 126: 111–134. DOI: 10.1007/s11120-014-0065-x.

Cardona T. 2016. Reconstructing the origin of oxygenic photosynthesis: do assembly and photoactivation recapitulate evolution? *Frontiers in Plant Science* 7: Article 257. DOI: 10.3389/fpls.2016.00257.

Cardona T. 2018. Early Archean origin of heterodimeric Photosystem I. *Heliyon* 4: Article e00549. DOI: 10.1016/j.heliyon.2018.e00548.

Cardona T. 2019. Thinking twice about the evolution of photosynthesis. *Open Biology* 9: Article 180246. DOI: 10.1098/rsob.180246.

Cardona T., Sánchez-Baracaldo P., Rutherford A.W. & Larkum A.W.D. 2019. Early Archaean origin of photosystem II. *Geobiology* 17: 127–150. DOI: 10.1111/gbi.12322.

Cassier-Chauvat C., Veauder T. & Chauvat F. 2016. Comparative genomics of DNA recombination and repair in cyanobacteria: biotechnological implications. *Frontiers in Microbiology* 7: Article 1809.

Checchetto V., Segalla A., Allorent G., La Rocca N., Leanza L., Giacometti G.M., Uozumi N., Finazzi G., Bergantino E. & Szabó I. 2012. Thylakoid potassium channel is required for efficient photosynthesis in cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America* 109: 11043–11048. DOI: 10.1073/pnas.1205960109.

Choi A.R., Shi L., Brown L.S. & Jung K.-H. 2014. Cyanobacterial light-driven proton pump, *Gloeobacter* rhodopsin: complementarity between rhodopsin-based energy production and photosynthesis. *PLOS One* 9: Article e110643. DOI: 10.1371/journal.pone.0110643.

Chrismas N.A.M., Anesio A. & Sánchez-Baracaldo P. 2015. Multiple adaptations to polar and alpine environments within cyanobacteria: a phylogenomic and Bayesian approach. *Frontiers in Microbiology* 6: Article 1070. DOI: 10.3389/fmicb.2015.01070.

Cockell C.S. & Raven J.A. 2007. Ozone and life on the Archaean Earth. *Philosophical Transactions of the Royal Society A* 365: 1889–1901. DOI: 10.1098/rsta.2007.2049.

Cockell C.S., Kaltenegger L. & Raven J.A. 2009. Cryptic photosynthesis–extrasolar planetary oxygen without a surface biological signature. *Astrobiology* 9: 623–626. DOI: 10.1089/ast.2008.0273.

Cork D.J., Garunas R. & Sajjad A. 1983. *Chlorobium limicola* forma *thiosulphatophilum*: biocatalyst in the production of sulfur and organic carbon from a gas stream containing $H_2S$ and $CO_2$. *Applied and Environmental Microbiology* 45: 913–918. DOI: 10.1128/AEM.45.3.913-918.1983.

D'Agostino P.M., Woodhouse, J.N., Liew, H.T., Luběk J., Pickford R., Wong H.L., Burns, B.P., Neilan B.A. 2019. Bioinformatic, phylogenetic, and chemical analysis of the UV-absorbing compounds scytonemin and mycosporine-like amino acids from the microbal mats of Shark Bay, Australia. *Environmental Microbiology* 21: 702–705. DOI: 10.1111/1462-2920-14517.

Damer B. & Deamer D. 2020. The hot spring hypothesis for an origin of life. *Astrobiology* 20: 429–452. DOI: 10.1089/ast.2019.2045.

Davis G.A. & Kramer D.M. 2020. Optimization of ATP synthase c-rings for oxygenic photosynthesis. *Frontiers in Plant Science* 10: Article 1778. DOI: 10.3389/fpls.2019.01778.

Davis G.A., Kanazawa A., Schöttler M.A., Kohzuma K., Froehlich J.E., Rutherford A.W., Satoh-Cruz M., Minhas D., Tietz S., Dhingra A. & Kramer D. 2016. Limitations to photosynthesis by proton motive force-induced photosystem II photodamage. *eLife* 5: Article e16921. DOI: 10.7554/eLife.16921.

Demoulin C.F., Lara Y.J., Cornet L., François C., Baurain D., Wilmotte A. & Javaux E.J. 2019. Cyanobacterial evolution: insights from the fossil records. *Free Radical Biology and Medicine* 140: 206–223. DOI: 10.1016/j.freeradbiomed.2019.05.007.

Dick G.J., Grim S.L. & Klatt J.M. 2018. Controls on $O_2$ production in cyanobacterial mats and implications for Earth's oxygenation. *Annual Review of Earth and Planetary Sciences* 46: 123–147. DOI: 10.1146/annurev-earth-082517-010035.

Du W., Jungbloets J.A., Hernández H.P., Bruggeman F.J., Hellingwerf K. J. & dos Santos F.B. 2016. Photonfluxstat: a method for light-limited batch cultivation of cyanobacteria at different, yet constant, growth rates. *Algal Research* 20: 118–125. DOI: 10.1016/j.algal.2016.10.004.

Duval S., Branscomb E., Trolard F., Bourrié G., Grauby O., Heresanu V., Schoepp-Cothenet B., Zuchan K., Russell M.J. & Nitschke W. 2020. On the why's and how's of clay minerals' importance in life's emergence. *Applied Clay Science* 195: Article 105737. DOI: 10.1016/j.clay.2020.105737.

Edwards D., Cherns L. & Raven J.A. 2015. Could land-based early photosynthesizing ecosystems have bioengineered the planet in mid-Palaeozoic times. *Palaeontology* 58: 803–837. DOI: 10.111/pala.12187.

Eisenhut M. 2019. Manganese homeostasis in Cyanobacteria. *Plants* 9: Article 18. DOI: 10.3390/plants9010018.

Elbert W., Weber B., Burrows S., Steinkamp J., Büdel B., Andreae M.O. & Pöschl U. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nature Geoscience* 5: 459–462. DOI: 10.1038/ngeo1486.

Eroglu S., van Zuilen M.A., Taubald H., Drost K., Wille M., Swanner E.D., Beukes N.J. & Schoenberg R. 2017. Depth-dependent $\delta^{13}C$ trends in platform and slope settings of the Campbellrand-Malmani carbonate platform and possible implications for Early Earth oxygenation. *Precambrian Research* 302: 122–139. DOI: 10.1016/j.precamres.2017.09.018.

Finke N., Simister R.L., O'Neil A.H., Nomosatryo S., Henny C., MacLean L.C., Canfield D.E., Kohauser K., Lalonde S.V., Fowle D.A. & Crowe S.A. 2019. Mesophilic microorganisms build terrestrial mats analogous to Precambrian microbial jungles. *Nature Communications* 10: Article 4323. DOI: 10.1038/s41467-019-11541-x.

Flynn K.J. 2009. Going for the slow burn: why should possession of a low maximum growth rate be advantageous for microalgae. *Plant Ecology and Diversity* 2: 179–189. DOI: 10.1080/17550870903207268.

Follmann H. & Brownson C. 2009. Darwin's warm little pond revisited: from molecules to the origin of life. *Naturwissenschaften* 96: 1265–1292. DOI: 10.1007/s00114-009-0602-1.

Frigaard N.-U., Voigt G.D. & Bryant D.A. 2002. *Chlorobium tepidum* mutant lacking bacteriochlorophyll *c* made by inactivation of the bchK gene, encoding bacteriochlorophyll *c* synthase. *Journal of Bacteriology* 184: 3368–3376. DOI: 10.1128/JB.184.12.3368-3376.2002.

Garcia-Pichel F., Lombard J., Soule T., Dunaj S., Wu S.H. & Wojciechowski M.F. 2019. Timing the evolutionary advent of cyanobacteria and the later great oxygenation event using gene phylogenies of a sunscreen. *mBio* 10: e00561–19. DOI: 10.1128/mBio.00561-19.

Grettenberger V.L., Sumner D.Y., Wall K., Brown C.T., Elsen J.A., MacKey T.J., Hawes I., Jospin G. & Jungblut A.D. 2020. A phylogenetically novel cyanobacterium most closely related to *Gloeobacter*. *The ISME Journal* 14: 2142–2152. DOI: 10.1038/s41396-020-0668-5.

Hagemann M. 2011. Molecular biology of cyanobacterial salt acclimation. *FEMS Microbiology Reviews* 35: 87–123. DOI: 10.1111/j.1574-6976.2010.00234.x.

Hamilton T.M. 2019. The trouble with oxygen: the ecophysiology of extant phototrophs and implications for the evolution of oxygenic photosynthesis. *Free Radical Biology and Medicine* 140: 233–249. DOI: 10.1016/j.freeradbiomed.2019.05.003.

Handley L.L. & Raven J.A. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell and Environment* 15: 965–985. DOI: 10.1111/j.1365-3040.1992.tb01650.x.

Hauska G., Schoedl T., Remigy H. & Tsiotis G. 2001. The reaction center of green sulfur bacteria. *Biochimica et Biophysica Acta* 1507: 260–277. DOI: 10.1016/S0005-2728(01)00200-6.

Heidrich J., Thurotte A. & Schneider D. 2017. Specific interaction of IMP30/Vipp1 with cyanobacterial and chloroplast membranes results in membrane remodeling and eventually in membrane fusion. *Biochimica et Biophysica Acta* 1859: 537–549. DOI: 10.1016/j.bbamem.2016.09.025.

Heinz S., Rast A., Shao L., Gutu A., Gügel I.L., Heyno E., Labs, M., Rengstl B., Viola S., Nowaczyk M.M. *et al.* 2016. Thylakoid membrane curvature in *Synechocystis* depends on CurT, a homolog of the granal CURVATURE THYLAKOID proteins. *The Plant Cell* 28: 2238–2260. DOI: 10.1105/tpc.16.00491.

Herdean A., Teardo E., Nilsson A.K., Pfeil B.E., Johansson O.N., Ünnep R., Nagy G., Zsiros O., Dana S., Solymosi K. *et al.* 2016. A voltage-dependent chloride channel fine-tunes photosynthesis in plants. *Nature Communications* 7: Article 11654. DOI: 10.1038/ncomms11654.

Herrmann A.J. & Gehringer M.M. 2019. An investigation into the effects of increasing salinity on photosynthesis in freshwater unicellular bacteria during the late Archean. *Geobiology* 17: 343–359. DOI: 10.1111/gbi.12339.

Hinterstoisser B. & Peschek G.A. 1987. Fluorimetric pH measurement in whole cells of dark aerobic and anaerobic cyanobacteria. *FEBS Letters* 217: 169–173. DOI: 10.1016/0014-5793(87)80657-9.

Homann N., Sansjofre P., Van Zuilen M., Heubeck C., Gong J., Killingsworth B., Foster I.S., Airo A., Van Kranendonk M.J., Ader M. & Lalonde S.V. 2018. Microbial life and biogeochemical cycling on land 3,220 million years ago. *Nature Geoscience* 11: 665–671. DOI: 10.1038/s41561-018-0190-9.

Huffert H.E. & Turner J.S. 1972. Double-diffusive convection and its implications for the temperature and salinity structure of the Ocean and Lake Vanda. *Journal of Physical Oceanography* 2: 456–461. DOI: 10.1175/1520-0485(1972)002<0456:DDCAII>2.0.CO;2.

Jahn M., Vialas V., Karlsen J., Maddalo G., Edfors F., Forsström B., Uhlén M., Käll L. & Hudson E.P. 2018. Growth of cyanobacteria is constrained by the abundance of light and carbon assimilation proteins. *Cell Reports* 25: 478–486. DOI: 10.1016/j.celrep.2018.09.040.

Jones H.E., Holland I.B. & Campbell A.K. 2002. Direct measurement of free $Ca^{2+}$ shows different regulation of $Ca^{2+}$ between the periplasm and the cytosol of *Escherichia coli*. *Cell Calcium* 32: 183–192. DOI: 10.1016/S0143416002001537.

Kamennaya N.A., Geraki K., Scanlan D. & Zubkov M.V. 2020. Accumulation of ambient phosphate into the periplasm of marine bacteria is proton motive force dependent. *Nature Communications* 11: 2642. DOI: 10.1038/s41467-020-16428-w.

Keren N., Kidd M.J., Penner-Hahn J.E. & Pakrasi H.B. 2002. A light-dependent mechanism of massive accumulation of manganese in the photosynthetic bacterium *Synechocystis* sp. PCC 6803. *Biochemistry* 41: 15085–15092. DOI: 10.1021/bi026892s.

Kihara S., Hartzler D.A. & Savikhin S. 2014. Oxygen concentration inside a functioning photosynthetic cell. *Biophysical Journal* 106: 1882–1889. DOI: 10.1016/j.bpj.2014.03.031.

Kitadai N. & Maruyama S. 2018. Origins of building blocks of life: a review. *Geoscience Frontiers* 9: 1117–1153. DOI: 10.1016/j.gsf.2017.07.007.

Kobayashi M., Katoh H. & Ikeuchi M. 2006. Mutations in a putative chloride efflux transporter gene suppress the chloride requirements of photosystem II in the cytochrome c550-deficient mutant. *Plant Cell Physiology* 47: 799–804. DOI: 10.1093/pcp/pcj052.

Koehler M.E, Buick R., Kipp N.A., Stücker E.E. & Zaloumis J. 2018. Transient surface oxygenation recorded in the ~2.66-Ga Jeerinah Formation, Australia. *Proceedings of the National Academy of Science* 115: 1711–1716. DOI: 10.1077/pnas.1720820115.

Koenig F. & Schmidt M. 1995. *Gloeobacter violaceus* — investigation of an unusual photosynthetic apparatus. Absence of the long-wavelength emission of photosystem I in 77 K fluorescence spectra. *Physiologia Plantarum* 94: 621–628. DOI: 10.1111/j.1399-3054.1995.tb00976.x.

Kowata H., Tochigi S., Takahashi H. & Kojima S. 2017. Outer membrane permeability of cyanobacterium *Synechocystis* sp. strain PCC 6803: studies of passive diffusion of small organic nutrients reveal the absence of classical porins and intrinsically low permeability. *Journal of Bacteriology* 199: Article e00371–17. DOI: 10.1128/JB.00371-17.

Koyama K., Suzuki H., Noguchi T., Akimoto S., Tsuchiya T. & Mimuro M. 2008. Oxygen evolution in the thylakoid-lacking cyanobacterium *Gloeobacter violaceus* PCC 7421. *Biochimica et Biophysica* 1777: 369–378. DOI: 10.1016/j.bbabio.2008.01.009.

Kuan D., Duff S., Posarc D. & Bi X. 2015. Growth optimization of *Synechococcus elongatus* PCC7942 in lab flasks and a 2-D photobioreactor. *The Canadian Journal of Chemical Engineering* 93: 640–647. DOI: 10.1002/cjce.22154.

Kugler A. & Dong H. 2019. Phyllosilcates as protective habitats of filamentous cyanobacteria *Leptolyngbya* against ultraviolet radiation. *PLOS One* 14: Article 0219616. DOI: 10.1371/journal.pone.0219616.

Kump, L.R., Junium, C., Arthur, M.A., Brasier, A., Fallick, A., Melezhik, V., Lepland, A., CČrne, A.E. & Luo, G. 2011. Isotopic evidence for maximum oxidation of organic matter following the Great Oxidation Event. *Science* 334: 1694–1696. DOI: 10.1126/science.1213999.

Lachapelle J., Bell G. & Colegrave N. 2015. Experimental adaptation to marine conditions by a freshwater alga. *Evolution* 69: 2662–2675. DOI: 10.1111/evo.12760.

Lea-Smith D.J., Bombelli P., Vasudevan R. & Howe C.J. 2010. Photosynthetic, respiratory, and extracellular electron transport pathways in cyanobacteria. *Biochimica et Biophysica Acta* 1857: 247–255. DOI: 10.1016/j.bbabio.2015.10.007.

Lehmer O.R., Catling D.C., Buick R., Brownlee D.E. & Newport S. 2020. Atmospheric $CO_2$ levels from 2.7 billion years ago inferred from

micrometeorite oxidation. *Science Advances* 6: eaay4644. DOI: 10.1126/sciadv.aay4644.

Leister D. 2018. Experimental evolution in photoautotrophic microorganisms as a means of enhancing chloroplast functions. *Essays in Biochemistry* 62: 77–184. DOI: 10.1042/EBC20170010.

Lenton T.M. & Daines S.J. 2017. Matworld – the biogeochemical effects of early life on land. *New Phytologist* 215: 531–537. DOI: 10.1111/nph.14338.

Lionard H., Péquin B., Lovejoy C. & Vincent W.F. 2012. Benthic cyanobacterial mats in the high Arctic: multi-layer structure and fluorescence responses to osmotic stress. *Frontiers in Microbiology* 3: Article 140. DOI: 10.3389/fmicb.2012.00140.

Liu H., Bidigare R.R., Laws E., Landry M.R. & Campbell L. 1999. Cell cycle and physiological characteristics of *Synechococcus* (WH7803) in chemostat culture. *Marine Ecology Progress Series* 189: 17–25. DOI: 10.3354/meps189017.

Liu L.-N. 2016. Distribution and dynamics of electron transport complexes in cyanobacterial thylakoid membranes. *Biochimica et Biophysica Acta* 1857: 256–265. DOI: 10.1016/j.bbabio.2015.11.010.

Loeks-Johnson B.M. & Cotner J.B. 2020. Upper Midwest lakes are supersaturated with $N_2$. *Proceedings of the National Academy of Science of the United States of America* 117: 17063–17067. DOI: 10.1073/pnas.1921689117.

Luimstra V.M., Schuurmans J.H., De Carvalho C.F.M., Matthijs H.C.P., Hellingwerf K.J. & Huisman J. 2019. Exploring the low photosynthetic efficiency of cyanobacteria in blue light using a mutant lacking phycobilisomes. *Photosynthesis Research* 141: 291–301. DOI: 10.1007/s11120-019-00630-z.

Lüttge U. 2011. Cyanobacteria: multiple stresses, desiccation tolerant photosynthesis and dinitrogen fixation. In: *Plant desiccation tolerance* (Ed. by U. Lüttge, E. Beck & D. Bartels), pp 23–43. Springer, Heidelberg, Germany.

Lyons T.W., Reinhard C.T. & Planavsky N. 2014. Evolution: a fixed-nitrogen fix in the early ocean? *Current Biology* 24: R276–R278. DOI: 10.1016/j.cub.2014.02.034.

Mahbub M., Hemm L., Yang Y., Kaur R., Carmen H., Engl C., Huokko T., Riediger M., Watanabe S., Liu L.N. *et al.* 2020. mRNA localization, reaction centre biogenesis and thylakoid membrane targeting in cyanobacteria. *Nature Plants* 6: 1179–1191. DOI: 10.1038/s41477-020-00764-2.

Mahinthichaichan P., Morris D.M., Wang Y., Jensen G.J. & Tajkhorshid E. 2018. Selective permeability of carboxysome shell pores to anionic molecules. *The Journal of Physical Chemistry B* 122: 9110–9118. DOI: 10.1021/acs.jpcb.8b06822.

Mareš J., Hrousek P., Kaňa R., Ventura S., Strunecký O. & Komárek J. 2013a. The primitive thylakoid-less cyanobacterium *Gloeobacter* is a common rock-dwelling organism. *PLOS One* 8: e66323. DOI: 10.1371/journal.pone.0066323.

Mareš J., Komárek J., Compère P. & Oren A. 2013b. Validation of the generic name *Gloeobacter* Rippka *et al.* 1974, Cyanophyceae. *Cryptoganie, Algologie* 34: 255–262. DOI: 10.7872/crya.v34.iss3.2013.255.

Mareš J., Komárek J., Compère P. & Oren A. 2013c. (2194) proposal to conserve the name *Gloeobacter violaceus* against *Aphanothece caldariorum, Gloeothece coerulea* and *Gloeothece linearis* (Cyanophyceae). *Taxon* 62: 1055. DOI: 10.12705/625.23.

Mareš J., Strunecky O., Bučinská L. & Wiedermannová J. 2019. Evolutionary patterns of thylakoid architecture in cyanobacteria. *Frontiers in Microbiology* 10: Article 277. DOI: 10.3389/fmicb.2019.00277.

McNevin D.B., Badger M.R., Whitney S.M., von Caemmerer S., Tcherkez G.G.B. & Farquhar G.D. 2007. Differences in carbon isotope discrimination of three variants of D-Ribulose-1,5-bisphosphate carboxylase/oxygenase reflect differences in their catalytic mechanisms. *The Journal of Biological Chemistry* 282: 36068–36076. DOI: 10.1074/jbc.M706274200.

Mendonça R., Müller R.A., Clow D., Verpoorter C., Raymond P., Tranvik L.J. & Sobek S. 2017. Organic carbon burial in global lakes and reservoirs. *Nature Communications* 8: Article 1694. DOI: 10.1038/s41467-017-01789-6.

Mimuro M., Tsuchiya T., Koyana K. & Peschek G.A. 2011. Bioenergetics in a primordial cyanobacterium *Gloeobacter violaceus* PCC 7421. In: *Bioenergetic processes of Cyanobacteria* (Ed. by G.A. Peschek, C. Obinger & G. Renger), pp 211–238. Springer, Heidelberg, Germany.

Mishra S.K., Shrivastava A., Maurya R.R., Patidar S.K., Haldar S. & Mishra S. 2012. Effect of light quality on the C-phycoerythrin production in marine cyanobacteria *Pseudanabaena* sp. isolated from the Gujarat coast, India. *Protein Expression and Purification* 81: 5–10. DOI: 10.1016/j.pep.2011.08.011.

Mloszewska A.M., Cole D.B., Planavsky N.J., Kappler A., Whitford D.S., Owttrim G.W. & Konhauser K.O. 2018. UV radiation limited the expansion of cyanobacteria in early marine photic environments. *Nature Communications* 9: Article 3088. DOI: 10.1038/s41467-018-05520-x.

Mulkidjanian A.Y., Koonin E.V., Makarova K.S., Mekhedev S.I., Sorokin A., Wolf Y.I., Dufresne A., Partensky S.I., Burd H., Kanadey D. *et al.* 2006. The cyanobacterial core genome and the origin of photosynthesis. *Proceedings of the National Academy of Science of the United States of America* 103: 13126–13131. DOI: 10.1073/pnas.0605709103.

Mullineaux C.W. 2014. Co-existence of photosynthetic and respiratory activities in cyanobacteria thylakoid membranes. *Biochimica et Biophysica Acta* 1837: 503–511. DOI: 10.1016/j.bbabio.2013.11.017.

Mullineaux C.W. & Liu L.-N. 2020. Membrane dynamics in phototrophic bacteria. *Annual Reviews of Microbiology* 74: 633–654. DOI: 10.1146/annurev-micro-020518-120134.

Mulo P., Sakurai I. & Aro E.-M. 2012. Strategies for *psbA* gene expression in cyanobacteria, green algae and higher plants: from transcription to PSII repair. *Biochimica et Biophysica Acta* 1817: 247–257. DOI: 10.1016/j.bbabio.2011.04.011.

Murphy D.J. 1982. The importance of non-planar bilayer regions in photosynthetic membranes and their stabilisation by galactolipids. *FEBS Letters* 150: 19–26. DOI: 10.1016/0014-5793(82)81297-0.

Nakov T., Boyko J.D., Alverson A.J. & Beaulieu J.M. 2017. Models with unequal transition rates favor marine origins of Cyanobacteria and photosynthetic eukaryotes. *Proceedings of the National Academy of Science of the United States of America* 114: E10606–E10607. DOI: 10.1073/pnas.1716692114.

Nelissen B., Van de Peer Y., Wilmotte A. & De Wachter R. 1995. An early origin of plastids within the cyanobacterial divergence is suggested by evolutionary trees based on complete 16S rRNA sequences. *Molecular Biology and Evolution* 12: 1166–1173. DOI: 10.1093/oxfordjournals.molbev.a040289.

Nguyen T.A., Brescic J., Vinyard D.J., Chandrasekar T. & Dismukes G.C. 2012. Identification of an oxygenic reaction center *psbADC* operon in the cyanobacterium *Gloeobacter violaceus* PCC 7421. *Molecular Biology and Evolution* 30: 35–38. DOI:10.1093/molbev/msr224.

Nicholls D.G. & Ferguson S.J. 2013. *Bioenergetics 4*. Elsevier, Academic Press, London, UK. 434 pp.

Nickelsen J. & Rengstl B. 2013. Photosystem II assembly: from cyanobacteria to plants. *Annual Review of Plant Biology* 64: 609–635. DOI: 10.1146/annurev-arplant-050312-120124.

Nielsen S.L. 2006. Size-dependent growth rates in eukaryotic and prokaryotic algae exemplified by green algae and cyanobacteria: comparison between unicells and colonial growth forms. *Journal of Plankton Research* 28: 489–498. DOI: 10.1093/plankt/fbi134.

Noffke N., Christian D., Wacey D. & Hazen R.M. 2013. Microbially induced sedimentary structures recording an ancient ecosystem in the *ca.* 3.48 billion year old Dresser Formation, Pilbara, Western Australia. *Astrobiology* 13: 1103–1124. DOI: 10.1089/ast.2013.1030.

Och, L.M. & Shields-Zhou, G.A. (2012) The Neoproterozoic oxygenation event: Environmental perturbations and biogeochemical cycling. *Earth-Science Reviews* 110: 26–57. DOI: 10.1016/j.earscirev.2011.09.004.

Ossa F.O., Hofmann A., Spangenberg J.E., Poulton S.W., Stüeken E.E., Schoenberg R., Eickmann B., Wille M., Butler M. & Bekker A. 2019. Limited oxygen production in the Mesoarchean ocean. *Proceedings of the National Academy of Science of the United States of America* 116: 6647–6652. DOI: 10.1073/pnas.1818762116.

Pade N., Compaoré J., Klähn S., Stal L.J. & Hagemann M. 2012. The marine cyanobacterium *Crocosphaera watsonii* WH8501 synthesizes

the compatible solute trehalose by a laterally acquired OtsAB fusion protein. *Environmental Microbiology* 14: 1261–1271. DOI: 10.1111/j.1462-2920.2012.02709.x.

Pade N., Michalik D., Ruth W., Belkin N., Hess W.R., Berman-Frank I. & Hagemann M. 2016. Trimethylated homoserine functions as the major compatible solute of the globally significant oceanic cyanobacterium *Trichodesmium*. *Proceedings of the National Academy of Science of the United States of America* 113: 13191–13196. DOI: 10.1073/pnas.1611666113.

Paiste K., Lephard A., Zerkel A.L., Kirsimae K., Kreitsman T., Mand K., Ramashkin A.E., Rychanekik K. & Prave A.R. 2020. Identifying global *vs* basinal controls on Palaeoproterozoic organic carbon and sulfur isotope records. *Earth Science Reviews* 207: Article 103239. DOI: 10.1016/j.earscirev.2020.103230.

Pathak J., Rajneesh, Singh P.R., Häder D.P. & Sinha R.P. 2019. UV-induced DNA damage and repair: a cyanobacterial perspective. *Plant Gene* 19: Article 100194. DOI: 10.1016/j.plgene.2019.100194.

Perrineau M.M., Zelzion E., Gross J., Price D.C., Boyd J. & Bhattacharya D. 2014. Evolution of salt tolerance in a laboratory reared population of *Chlamydomonas reinhardtii*. *Environmental Biology* 16: 1755–1766. DOI: 10.1111/1462-2920.12372.

Pogoryelov D., Reichen C., Klyszejko A.-L., Brunisholz R., Muller D.J., Dimroth P. & Meier T. 2007. The oligomeric state of c rings from cyanobacterial F-ATP synthases varies from 13 to 15. *Journal of Bacteriology* 189: 5895–5902. DOI: 10.1128/JB.00581-07.

Ponce-Toledo R., Deschamps P., López-García P., Zivanovic Y., Benzerara K. & Moreira D. 2017. An early-branching freshwater cyanobacterium at the origin of plastids. *Current Biology* 27: 386–391. DOI: 10.1016/j.cub.2016.11.056.

Popall R.M., Bolhuis H., Muyzer G. & Sánchez-Román M. 2020. Stromatolites as biosignatures of atmospheric oxygenation: carbonate biomineralization and UV-C resilience in a *Geitleronema* sp.-dominated culture. *Frontiers in a Microbiology* 11: Article 948. DOI: 10.3389/fmicb.2020.00948.

Popovic S., Nikolić N., Jovanović J., Predojević D., Trbojević I., Manić L. & Dimic G.S. 2019. Cyanobacterial and algal abundance and biomass in cave biofilms and relation to environmental and biofilm parameters. *International Journal of Speleology* 48: 49–61. DOI: 10.5038/1827-806X.48.1.2224.

Post A.F., Loogman J.G. & Mur L.R. 1986. Photosynthesis, carbon flows and growth of *Oscillatoria agardhii* Gomont in environments with a periodic supply of light. *Journal of General Microbiology* 132: 2129–2136. DOI: 10.1099/00221287-132-8-2129.

Potts M., Slaughter S.M., Hunneke F.-U., Garst J.F. & Helm R.F. 2005. Desiccation tolerance of prokaryotes: application of principles to human cells. *Integrative and Comparative Biology* 45: 800–809. DOI: 10.1093/icb/45.5.800.

Pushkareva E., Pessi I.S., Wilmotte A. & Elster J. 2015. Cyanobacterial community composition in Arctic soil crusts at different stages of development. *FEMS Microbiology Evolution* 91: fiv 143. DOI: 10.1093/femsec/fiv143.

Rae B.D., Long B.M., Badger M.R. & Price G.D. 2013. Functions, compositions, and evolution of the two types of carboxysomes: polyhedral microcompartments that facilitate $CO_2$ fixation in cyanobacteria and some proteobacteria. *Microbiology and Molecular Biology Reviews* 77: 357–379. DOI: 10.1128/MMBR.00061-12.

Rast A., Schaeffer M., Albert S., Wan W., Pfeffer S., Beck F., Plitzko J.M., Nickelsen J. & Engel B.D. 2019. Biogenetic regions of cyanobacterial thylakoids form contact sites from with the plasma membrane. *Nature Plants* 5: 436–446. DOI: 10.1038/s41477-019-0399-7.

Rastogi R.P., Sinha R.P., Moh S.H., Lee T.K., Kottuparambil S., Ki Y.-S., Rhee J.-S., Choi E.-M., Brown M.T., Häder D.-P. & Han T. 2014. Ultraviolet radiation and cyanobacteria. *Journal of Photochemistry and Photobiology B: Biology* 141: 154–169. DOI: 10.1016/j.jphotobiol.2014.09.020.

Raven J.A. 1984. A cost-benefit analysis of photon absorption by photosynthetic unicells. *New Phytologist* 98: 593–625. DOI: 10.1111/j.1469-8137.1984.tb04152.x.

Raven J.A. 2009. Functional evolution of photochemical energy transformations in oxygen-producing organisms. *Functional Plant Biology* 36: 505–515. DOI: 10.1071/FP09087.

Raven J.A. 2017. Chloride: essential micronutrient and multifunctional beneficial ion. *Journal of Experimental Botany* 68: 359–367. DOI: 10.1093/jxb/erw421.

Raven J.A. 2018. How long have photosynthetic organisms been aggregating soils? *New Phytologist* 219: 1139–1141. DOI: 10.1111/nph.15309.

Raven J.A. 2020. Chloride involvement in the synthesis, functioning and repair of the photosynthetic apparatus *in vivo*. *New Phytologist* 227: 334–342. DOI: 10.1111/nph.16541.

Raven J.A. & Beardall J. 2016. The ins and outs of $CO_2$. *Journal of Experimental Botany* 67: 1–13. DOI: 10.1093/jxb/erv451.

Raven J.A. & Beardall J. 2020. Energizing the plasmalemma of marine photosynthetic organisms: the role of primary active transport. *Journal of the Marine Biological Association United Kingdom* 100: 333–346. DOI: 10.1017/S0025315420000211.

Raven J.A., Beardall J. & Giordano M. 2014. Energy costs of $CO_2$ concentrating mechanisms in aquatic organisms. *Photosynthesis Research* 121: 111–124. DOI: 10.1007/s11120-013-9962-7.

Raven J.A., Beardall J. & Sánchez-Baracaldo P. 2017. The possible evolution and future of $CO_2$-concentrating mechanisms. *Journal of Experimental Botany* 68: 3701–3716. DOI: 10.1093/jxb/erx110.

Raven J.A., Johnston A.M., Kübler J.E., Korb R., McInroy S.G., Handley L.L., Scrimgeour C.M., Walker D.I., Beardall J., Vanderklift M. *et al.* 2002. Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. *Functional Plant Biology* 29: 355–378. DOI: 10.1071/PP01201.

Raven J.A., Knight C. & Beardall J. 2019. Cell size has gene expression and biophysical consequences for cellular function. *Perspectives in Phycology* 6: 91–96. DOI: 10.1127/pip/2019/0086.

Raven J.A., Suggett D.J. & Giordano M. 2020. Inorganic carbon concentrating mechanisms in free-living and symbiotic dinoflagellates and chromerids. *Journal of Phycology* 56: 1377–1397. DOI: 10.1111/jpy.13050.

Rawyler A., Unitt M.D., Giroud C., Davies H., Mayor J.P., Harwood J.L. & Siegenthaler P.-A. 1987. The transmembrane distribution of galactolipids in chloroplast thylakoids is universal in a wide variety of temperate climate plants. *Photosynthesis Research* 11: 3–13. DOI: 10.1007/BF00117669.

Retallak G.J., Krimsley D.H., Fischer R., Razink J.J. & Langworthy K.A. 2016. Archean coastal-plain paleosols and life on land. *Gondwana Research* 40: 1–20. DOI: 10.1016/j.gr.2016.08.003.

Rexroth S., Mullineaux C.W., Ellinger D., Sendtko E., Rögner M. & Koenig F. 2011. The plasma membrane of the cyanobacterium *Gloeobacter violaceus* contains segregated bioenergetic domains. *The Plant Cell* 23: 2379–2390. DOI: 10.1105/tpc.111.085779.

Rippka R., Waterbury J. & Cohen-Bazire G. 1974. A cyanobacterium which lacks thylakoids. *Archives of Microbiology* 100: 419–436. DOI: 10.1007/BF00446333.

Ritchie R.J. 1991. Membrane potential and pH control in the cyanobacterium *Synechococcus* R-2 (*Anacystis nidulans*) PCC 7942. *Journal of Plant Physiology* 137: 409–418. DOI: 10.1016/S0176-1617(11)80309-3.

Ritchie R.J. 1992. The cyanobacterium *Synechococcus* R-2 (*Anacystis nidulans, S. leopolensis*) PCC7942 has a sodium-dependent chloride transporter. *Plant Cell and Environment* 15: 163–177. DOI: 10.1111/j.1365-3040.1992.tb01470.x.

Ruan Z. & Giordano M. 2017. The use of $NH_4^+$ rather than $NO_3^-$ affects cell stoichiometry, C allocation, growth in the cyanobacterium *Synechococcus* UTEX LB 2380, only when energy is limiting. *Plant, Cell and Environment* 40: 227–236. DOI: 10.1111/pce.12858.

Sánchez-Baracaldo P. 2015. Origin of marine planktonic cyanobacteria. *Scientific Reports* 5: 17418. DOI: 10.1038/srep17418.

Sánchez-Baracaldo P. & Cardona T. 2020. On the origin of oxygenic photosynthesis and Cyanobacteria. *New Phytologist* 225: 1440–1446. DOI: 10.1111/nph.16249.

Sánchez-Baracaldo P., Ridgwell A. & Raven J.A. 2014. A neoproterozoic transition in the marine nitrogen cycle. *Current Biology* 24: 652–657. DOI: 10.1016/j.cub.2014.01.041.

Sánchez-Baracaldo P., Raven J.A., Pisani D. & Knoll A.H. 2017a. Early photosynthetic eukaryotes inhabited low-salinity habitats. *Proceedings*

*of the National Academy of Science of the United States of America* 114: E7737–E7745. DOI: 10.1073/pnas.1620089114.

Sánchez-Baracaldo P., Bianchini G., Huelsenbeck J.P., Raven J.A., Pisani D. & Knoll A.H. 2017b. Reply to Nakov *et al.*: model choice requires biological insight when studying the ancestral habitat of photosynthetic eukaryotes. *Proceedings of the National Academy of Science of the United States of America* 114: E10608–E10609. DOI: 10.1073/pnas.1717417114.

Sánchez-Baracaldo P., Bianchini G., Di Cesare A., Callieri C. & Chrismas N.A.M. 2019. Insights into the evolution of picocyanobacteria and phycoerythrin genes (*mpeBA* and *cpeBA*). *Frontiers in Microbiology* 10: 45. DOI: 10.3389/fmicb.2019.00045.

Sand-Jensen K. 2014. Ecophysiology of gelatinous *Nostoc* colonies: unprecedented slow growth and survival in resource-poor and harsh environments. *Annals of Botany* 114: 17–33. DOI: 10.1093/aob/mcu085.

Saw J.H.W., Schatz M., Brown M.V., Kunkel D.D., Foster J.S., Shick H., Cristensen S., Hou S., Wan X. & Donachie S.P. 2013. Cultivation and complete genome sequencing of *Gloeobacter kilauensis* sp. nov. from a lava cave in Kīlauea Caldera, Hawai'i. *PLOS One* 8: e76376. DOI: 10.1371/journal.pone.0076376.

Scanlan D.J., Ostrowski M., Mazard S., Dufresne A., Garczarek L., Hess W.R., Post A.F., Hagemann M., Paulsen I. & Partensky F. 2009. Ecological genomics of marine picocyanobacteria. *Microbiology and Molecular Biology Reviews* 73: 249–299. DOI: 10.1128/MMBR.00035-08.

Schad M., Kohnhauser K.O., Sánchez-Baracaldo P., Koppler A. & Bryce C. 2019. How did the evolution oxygenic photosynthesis influence the temporal and spatial development of the microbial iron cycle on ancient Earth? *Free Radical Biology and Medicine* 140: 154–166. DOI: 10.1016/j.freeradbiomed.2019.07.014.

Schirrmeister B.E., de Vos J.M., Antonelli A. & Bagheri H.C. 2013. Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event. *Proceedings of the National Academy of Science of the United States of America* 110: 1791–1796. DOI: 10.1073/pnas.1209927110.

Schirrmeister, B.E., Gugger, M. & Donoghue, P.C.J. 2015. Cyanobacteria and the Great Oxidation Event: evidence from genes and fossils. *Palaeontology* 5: 769–785. DOI: 10.1111/pala.12129.

Schirrmeister B.E., Sanchez-Baracaldo P. & Wacey D. 2016. Cyanobacterial evolution during the Precambrian. *International Journal of Astrobiology* 15: 187–204. DOI: 10.1017/S1473550415000579.

Schneider S. & Jürgens U.J. 1991. Cell wall and sheath constituents of the cyanobacterium *Gloeobacter violaceus*. *Archives of Microbiology* 156: 312–318. DOI: 10.1007/BF00263004.

Selão T.T., Zhang L., Ariöz C., Wieslander Å. & Norling B. 2014. Subcellular localization of monoglucosyldiacylglycerol synthase in *Synechocystis* sp. PCC6803 and regulation by lipid environment. *PLOS One* 9: e88153. DOI: 10.1371/journal.pone.0088153.

Selstam E. & Campbell B. 1996. Membrane lipid composition of the unusual cyanobacterium *Gloeobacter violaceus* PCC7431, which lacks sufoquinovosyl diacylglycerol. *Archives of Microbiology* 195: 132–135. DOI: 10.1007/s002030050367.

Shih P.M., Occhialini A., Cameron J.C., Andralojc P.J., Parry M.A.J. & Kerfeld C.A. 2016. Biochemical characterization of predicted Precambrian RuBisCO. *Nature Communications* 7: Article 10382. DOI: 10.1038/ncomms10382.

Sicora C.I., Brown C.M., Cheregi O., Vass I. & Campbell D.A. 2008. The psbA gene family responds differentially to light and UVB stress in *Gloeobacter violaceus* PCC 7421, a deeply divergent cyanobacterium. *Biochimica et Biophysica Acta Bioenergetics* 1777: 130139. DOI: 10.1016/j.bbabio.2007.09.001.

Siegenthaler P.-A., Sutter J. & Rawyler A. 1988. The transmembrane distribution of galactolipids in spinach thylakoid inside-out vesicles is opposite to that found in intact thylakoids. *FEBS Letters* 228: 94–98. DOI: 10.1016/0014-5793(88)80592-1.

Six C., Thomas J.C., Brahamsha B., Lemoine V. & Partensky F. 2004. Photophysiology of the marine cyanobacterium *Synechocystis* sp. WH8102, a new model organism. *Aquatic Microbial Ecology* 35: 17–29. DOI: 10.3354/ame035017.

Sosik H.N., Olson R.J., Neubert M.G. & Shalapyonok A. 2003. Growth rates of coastal photoplankton from time-series measurements with a submersible flow cytometer. *Limnology and Oceanography* 48: 1756–1765. DOI: 10.4319/lo.2003.48.5.1756.

Stengel A., Gugel L.L., Hilger D., Rengstl B., Jung H. & Nickelsen J. 2012. Initial steps of photosystem II de novo assembly and preloading with manganese take place in biogenesis centers in *Synechocystis*. *The Plant Cell* 24: 660–675. DOI: 10.1105/tpc.111.093914.

Stueken E.E., Buick R., Anderson R.E., Baross J.A., Planavsky N.J. & Lyons T.W. 2017. Environmental niches and metabolic diversity in Neoarchean lakes. *Geobiology* 15: 767–783. DOI: 10.1111/gbi.12251.

Sumner D.Y., Hawes I., MacKey T.J., Jungblutt A.D. & Doran P.T. 2015. Antarctic microbial mats: a modern analogue for Archean lacustrine oxygen oases. *Geology* 43: 887–890. DOI: 10.1130/G36966.1.

Sumner D.Y., Jungblott A.D., Hawes I., Anderson D.T., MacKey T.J. & Wall K. 2016. Growth of elaborate pinnacles in Lake Vanda, Antarctica. *Geobiology* 14: 556–574. DOI: 10.1111/gbi.12188.

Sutter M., Laughlin T.G., Sloan N.B., Serwas D., Davies K.M. & Kerfeld C.A. 2019. Structure of a synthetic β-carboxysome shell. *Plant Physiology* 181: 1050–1058. DOI: 10.1104/pp.19.00885.

Tamura D., Saito K. & Ishikata H. 2020. Acquirement of water-splitting ability and alteration of the charge-separation mechanism in photosynthetic reaction centers. *Proceedings of the National Academy of Science of the United States of America* 117: 16373–16382. DOI: 10.1073/pnas.2000895117.

Theis J., Niemeyer J., Schmollinger S., Ries F., Rütgers M., Gupta T.K., Sommer F., Muranaka L.S., Venn B., Schultz-Raffelt M. *et al.* 2018. VIPP2 interacts with VIPP1 and HSP22E/F at chloroplast membranes and modulates a retrograde signal for HSP22E/F gene expression. *Plant Cell and Environment* 43: 1212–1229. DOI: 10.1111/pce.13732.

Thomas P.J., Boller A.J., Satagopan S., Tabita R.F., Cavanaugh C.M. & Scott K.M. 2018. Isotope discrimination by form IC RubisCo from *Ralstonia eutropha* and *Rhodobacter sphaeroides*, metabolically versatile members of 'Proteobacteria' from aquatic and soil habitats. *Environmental Microbiology* 21: 72–80. DOI: 10.1111/1462-2920.14423.

Thomazo C., Couradeau E., Giraldo-Silva A., Martin-Carbonne J., Brayard A., Homnann M., Sansjofre P., Lalande S.V. & Garcia-Pichel F. 2020. Biological soil crusts as modern analogs for the Archean continental biosphere: insights from carbon and nitrogen isotopes. *Astrobiology* 20: 815–819. DOI: 10.1089/ast.2019.2144.

Thornton L.E., Ohkawa H., Roose J.L., Kashino Y., Keren N. & Pakrasi H.B. 2004. Homologs of plant PsbP and PsbQ proteins are necessary for regulation of photosystem II proteins in the cyanobacterium *Synechocystis* 6803. *Plant Cell* 16: 2164-2175. DOI: 10.1105/tpc.104.023515.

Toner J.D. & Catling D.C. 2020. A carbonate-rich lake solution to the phosphate problem of the origin of life. *Proceedings of the National Academy of Science of the United States of America* 117: 883–888. DOI: 10.1073/pnas.1916109117.

Uyeda J.C., Harmon L.J. & Blank C.E. 2016. A comprehensive study of cyanobacterial morphological and ecological evolutionary dynamics through deep geologic time. *PLOS One* 11: e0162539. DOI: 10.1371/journal.pone.0162539.

Vinyard D.J., Badshah S.L., Riggio M.R., Kaur D., Fanguy A.R. & Gunner M.R. 2019. Photosystem II oxygen-evolving complex photoassembly displays an inverse H/D isotope effect under chloride-limiting conditions. *Proceedings of the National Academy of Science of the United States of America* 116: 18917–18922. DOI: 10.1073/pnas.1910231116.

Vogt A., Wietek J. & Hegemann P. 2013. *Gloeobacter* rhodopsin, limitation of proton pumping at high electrochemical load. *Biophysical Journal* 196: 2055–2063. DOI: 10.1016/j.bpj.2013.08.031.

Vothknecht L.C. & Westhoff P. 2001. Biogenesis and origin of thylakoid membranes. *Biochimica et Biophysica Acta* 1541: 91–101. DOI: 10.1016/S0167-4889(01)00153-7.

Watanabe Y., Martini J.E.J. & Ohmoto H. 2000. Geochemical evidence for terrestrial ecosystems 2.6 billion years ago. *Nature* 408: 574–577. DOI: 10.1038/35046052.

Westphal S., Heins L., Soll J. & Vothknecht U.C. 2001. *Vipp1* deletion mutant of *Synechocystis*: a connection between bacterial phage shock and thylakoid biogenesis? *Proceedings of the National Academy of Science of the United States of America* 98: 4242–4248. DOI: 10.1073/pnas.061501198.

Williams L., Loewen-Schneider K., Maier S. & Budel B. 2016. Cyanobacterial diversity in western European biological soil crusts along a latitudinal gradient. *FEMS Microbiology Ecology* 92: Article fiw157. DOI: 10.1093/femsec/fiw157.

Willmeth D.T., Corsetti F.A., Beukes N.J., Awramik S.M., Petryshyn V., Spear J.R. & Celestian A.J. 2019. Neoarchean (2.7 Ga) lacustrine stromatolite deposits in the Hartbeesfontein Basin, Ventersdorp Supergroup, South Africa: implications for oxygen oases. *Precambrian Research* 320: 291–302. DOI: 10.1016/j.precamres.2018.11.009.

Yu J., Liberton M., Clifton P.F., Head R.D., Jacobs J.M., Smith R.D., Kappenaal D.W., Brand J.J. & Pakraso H.B. 2015. *Synechococcus elongatus* UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and $CO_2$. *Scientific Reports* 5: Article 8132. DOI: 10.1038/srep08132.

Zavřel T., Faizi M., Loureiro C., Poschmann G., Stühler K., Sinetova M., Zorina A., Steuer R. & Červený J. 2019. Quantitative insights into the cyanobacterial cell economy. *eLife* 8: Article e42508. DOI: 10.7554/eLife.42508.

Zerkle A.L., Claire M.W., Domagal-Goldman S.D., Farquhar J. & Poulton S. W. 2012. A bistable organic-rich atmosphere on the Neoarchean Earth. *Nature Geosciences* 5: 359–363. DOI: 10.1038/ngeo1425.